02

PATENT COOPERATION TREATY

PCT

09/743682

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
586.02-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB 00/00673	19/05/2000	20/05/1999
Applicant		
SAATCIOGLU, Fahri		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☒ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 00/00673

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim(s) 27-28, 30-34, 36-38 (as far as in vivo methods are concerned) and claims 29 and 35 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-38

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The ISA has discovered an inconsistency between the numbering of the different sequences in the Description (including the claims) and the Sequence Listing. Indeed, the Description refers to the polypeptides corresponding to SEQ IDs 1 to 7 as SEQ IDs 8 to 14 respectively whereas these polypeptides correspond to SEQ IDs 15 to 21 in the Sequence Listing. The opposite is true for the RNA sequences corresponding to SEQ IDs 1 to 7, which are numbered 15 to 21 in the Description and 8 to 14 in the Sequence Listing.
For the purpose of the search, the ISA has adopted the numbering as defined in the Sequence Listing.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-38

1.1. Claims: 1-2,15-16 (totally) and claims 11-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 1, the encoded polypeptide SEQ ID 15, variants thereof, and their uses in methods for detecting neoplastic cells.

1.2. Claims: 3-4,17-18 (totally) and claims 11-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 2, the encoded polypeptide SEQ ID 16, variants thereof, and their uses in methods for detecting neoplastic cells.

1.3. Claims: 5-6,19-20 (totally) and claims 11-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 3, the encoded polypeptide SEQ ID 17, variants thereof, and their uses in methods for detecting neoplastic cells.

1.4. Claims: 7,21 (totally) and claims 13-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 4, the encoded polypeptide SEQ ID 18, variants thereof, and their uses in methods for detecting neoplastic cells.

1.5. Claims: 8,22 (totally) and claims 13-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 5, the encoded polypeptide SEQ ID 19, variants thereof, and their uses in methods for detecting neoplastic cells.

1.6. Claims: 9,23 (totally) and claims 13-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 6, the encoded polypeptide SEQ ID 20, variants thereof, and their uses in methods for detecting neoplastic cells.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1.7. Claims: 10,24 (totally) and claims 13-14, 25-38 (all partially)

A polynucleotide comprising SEQ ID 7, the encoded polypeptide SEQ ID 21, variants thereof, and their uses in methods for detecting neoplastic cells.

2. Claims: 39-44

A method for identifying differentially expressed genes in a target tissue.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 00/00673

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C12Q1/68 G01N33/68 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 37418 A (CORIXA CORP) 27 August 1998 (1998-08-27)	1,2,11, 13-16, 25-38
Y	page 31, line 6 - line 11 SEQ IDs 171 to 178	1-38
	claims	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2000	01. 02. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ANDRES S.M.

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/IB 00/00673

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETER S NELSON ET AL: "Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 6, 16 March 1999 (1999-03-16), pages 3114-3119, XP002141959 ISSN: 0027-8424 cited in the application	15
Y	the whole document	

P,X	STEPHENSON S A ET AL: "Localization of a new prostate-specific antigen-related serine protease gene, KLK4, is evidence for an expanded human kallikrein gene famly cluster on chromosome 19q13.3-13.4" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, 13 August 1999 (1999-08-13), pages 23210-23214, XP002141960 the whole document	1-38 15

P,X	GEORGE M YOUSEF ET AL: "Prostase/KLK-L1 is a new member of the human Kallikrein gene family, is espressed in prostate and breast tissues, and is hormonally regulated" CANCER RESEARCH, vol. 59, no. 17, 1 September 1999 (1999-09-01), pages 4252-4256, XP002141958 ISSN: 0008-5472 the whole document	15

P,X	EP 0 936 270 A (BASF AG) 18 August 1999 (1999-08-18) the whole document	15

P,A	WO 99 67384 A (SPRINZAK EINAT A ;INCYTE PHARMA INC (US); KLINGLER TOD M (US); VOL) 29 December 1999 (1999-12-29) abstract SEQ ID 3 (clone 1816556) page 14, last line -page 20 claims	8,9,13, 14,22, 23,25-38

P,X	WO 00 04149 A (CORIXA CORP) 27 January 2000 (2000-01-27) SEQ IDs 326 to 331, 171 to 176, and 225. page 56, line 26 -page 58	1,2,11, 13-16, 25-38

	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 00/00673

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	KORKMAZ, K. ET AL.: "An efficient procedure for cloning hormone-responsive genes from a specific tissue." DNA AND CELL BIOLOGY, vol. 19, no. 8, August 2000 (2000-08), pages 499-506, XP000953240 ISSN: 1044-5498	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 00/00673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9837418 A	27-08-1998	AU 6536898 A	09-09-1998
		BR 9807734 A	31-10-2000
		EP 0972201 A	19-01-2000
		ZA 9801536 A	08-01-1999
EP 0936270 A	18-08-1999	DE 19805633 A	19-08-1999
		JP 2000023678 A	25-01-2000
WO 9967384 A	29-12-1999	AU 4823599 A	10-01-2000
WO 0004149 A	27-01-2000	AU 5314899 A	07-02-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71711 A3

(51) International Patent Classification[7]: C12N 15/12, C07K 14/47, C12Q 1/68, G01N 33/68, C12N 15/10

(21) International Application Number: PCT/IB00/00673

(22) International Filing Date: 19 May 2000 (19.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/135,325 20 May 1999 (20.05.1999) US
60/135,333 20 May 1999 (20.05.1999) US

(71) Applicant and
(72) Inventor: SAATCIOGLU, Fahri [TR/NO]; University Of Oslo, Biotechnology Center Of Oslo, Gaustadalleen 21, P.O. Box 1125, N-1317 Blindern (NO).

(74) Agent: SAATCIOGLU, Fahri; Fish & Associates, LLP, Suite 706, 1440 N. Harbor Boulevard, Fullerton, CA 92835 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

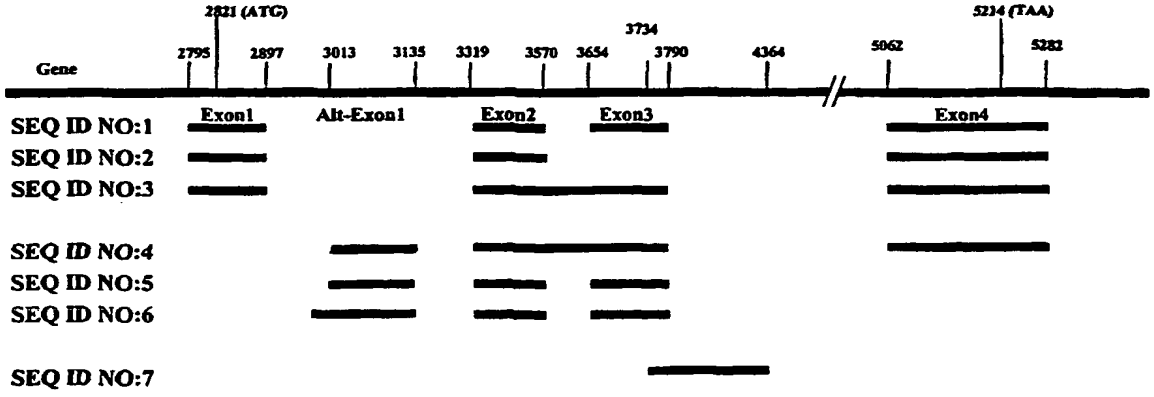
Published:
— *with international search report*
— *with amended claims*

(88) Date of publication of the international search report: 12 July 2001

Date of publication of the amended claims: 2 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIFFERENTIALLY EXPRESSED GENES IN PROSTATE CANCER



(57) Abstract: SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 encode an intracellular protein that is expressed in prostate epithelial cells in a hormone dependent manner. Encoded proteins SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 have predominantly perinuclear, nuclear and predominantly nuclear location localization within a cell, respectively. In contemplated methods of detecting a neoplastic cell in a system, a predetermined amount of at least one of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, or at least one of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 is correlated with the presence of a neoplastic cell and detected within the system employing specific binding of a labeled probe. In a method of identifying differentially expressed genes, a tissue specific array of cDNA prepared by suppression subtractive hybridization is arranged on a solid phase. Two nucleic acid preparations are individually hybridized with the array, wherein the first and second nucleic acid preparations are prepared from treated and untreated target tissue. A comparison of the hybridization patterns reveals differentially expressed genes.

WO 00/71711 A3

Attorney's Docket No. **586.02-PCT**

IN THE INTERNATIONAL BUREAU OF WIPO

International Application Number	International Filing Date	Earliest International Priority Date Claimed
PCT/IB00/00673	**19 May 2000**	**20 May 1999**

Title Of Invention: **Differentially Expressed Genes in Prostate Cancer**

Applicant(s): **SAATCIOGLU, Fahri**

European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk

VERIFIED CERTIFICATION OF MAILING DATE
(INTERNATIONAL APPLICATION (37 CFR 1.10(c))

I hereby declare that I deposited, with DHL Worldwide Express, addressed to " European Patent Office P.B. 5818 Patentlaan 2NL-2280 HV Rijswijk" the following papers:

Letter for PCT Article 19 Amendments (9 pages)
and Substitute Claims Sheets (4 pages)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: 18 Dec 2000

Fahri Saatcioglu

AMENDED CLAIMS
[received by the International Bureau on 4 January 2001 (04.01.01);
original claims 1-44 replaced by amended claims 1-32 (4 pages)]

1. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:3, wherein the nucleotide sequence encodes an intracellular protein.

2. The polynucleotide of claim 1 wherein the intracellular protein has a predominantly nuclear localization in a cell.

3. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:4, wherein the nucleotide sequence encodes an intracellular protein.

4. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:7, wherein the nucleotide sequence encodes an intracellular protein.

5. The polynucleotide of any one of claim 1, 3, or 4 wherein the polynucleotide is expressed *in vivo* in a prostate cancer cell.

6. The polynucleotide of any one of claims 1, 3, or 4 wherein the expression of the polynucleotide is dependent on at least one of an androgen, a progesterone, an estrogen, and a glucocorticoid.

7. A polynucleotide having at least 90% identity to at least one of SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

8. A polynucleotide having at least 95% identity to at least one of SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO:10, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

10. The polypeptide of claim 9 wherein the polypeptide has a predominantly nuclear localization in a cell.

11. A polypeptide comprising the amino acid sequence of SEQ ID NO:11, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

12. A polypeptide comprising the amino acid sequence of SEQ ID NO:14, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

13. A polypeptide having at least 90% homology to at least one of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

14. A polypeptide having at least 95% homology to at least one of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

15. A method of detecting a neoplastic cell, comprising:
correlating a predetermined quantity of an RNA comprising at least one of SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:21 in a cell containing system with a presence of a neoplastic cell, wherein the RNA encodes an intracellular polypeptide; and
detecting at least the predetermined quantity of the RNA in the system.

16. The method of claim 15 wherein the neoplastic cell is a prostate cancer cell.

17. The method of claim 15 wherein the system is a mammal.

18. The method of claim 15 wherein the step of detecting includes hybridization of a probe to at least one of the SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:21.

19. The method of claim 18 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

20. The method of claim 18 wherein at least one nucleotide is enzymatically coupled to the probe while the probe is hybridized to the at least one of the SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:21.

21. A method of detecting a neoplastic cell, comprising:
correlating a predetermined quantity of an intracellular polypeptide comprising at least one of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:14 in a cell containing system with a presence of a neoplastic cell; and

detecting at least the predetermined quantity of the intracellular polypeptide in the system.

22. The method of claim 21 wherein the neoplastic cell is a prostate cancer cell or a breast cancer cell.

23. The method of claim 21 wherein the system is a mammal.

24. The method of claim 21 wherein the step of detecting includes specifically binding of a probe to the polypeptide.

25. The method of claim 24 wherein the probe is selected from the group consisting of an antibody, an antibody fragment, a natural ligand of the polypeptide, and a synthetic ligand of the polypeptide.

26. The method of claim 24 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

27. A method of identifying differentially expressed genes in a target tissue, comprising:

providing a target tissue-specific cDNA library having a plurality of tissue-specific genes, wherein the tissue-specific genes are obtained by suppression subtractive hybridization;

immobilizing a predetermined quantity of tissue-specific genes on a solid phase to form a tissue-specific cDNA array;

hybridizing a first nucleic acid preparation to a first tissue-specific cDNA array to create a first hybridization pattern, wherein the first preparation is prepared from the target tissue without previously exposing the target tissue to a compound;

hybridizing a second nucleic acid preparation to a second tissue-specific cDNA array to create a second hybridization pattern, wherein the second preparation is prepared from the target tissue after previously exposing the target tissue to a compound; and

comparing the first and the second hybridization pattern to identify differentially expressed genes.

28. The method of claim 27 wherein the target tissue comprises prostate tissue.

29. The method of claim 28 wherein the prostate tissue comprises prostate cancer cells.

30. The method of claim 27 wherein the solid phase comprises a membrane.

31. The method of claim 27 wherein the compound comprises a hormone.

32. The method of claim 27 wherein at least one of the first and second nucleic acid preparations is radiolabeled and wherein the step of comparing comprises phosphorimaging.

Practitioner's Docket No. 586.02-PCT

IN THE INTERNATIONAL BUREAU (WIPO)

International Application Number	International Filing Date	International Earliest Priority Date
PCT/IB00/00673	19 May 2000	20 May 1999

Title of Invention: Differentially Expressed Genes In Prostate Cancer
Applicant: Saatcioglu, Fahri

European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk

LETTER FOR PCT ARTICLE 19 (PCT SECTION 205)

1. Applicant herewith submits replacement sheets numbered **26-30** to replace sheets numbered **26-31** originally filed for this application.

2. In respect of each claim appearing in the international application as originally filed, the following claim(s) is/are:

(i) unchanged: claim(s) **5, 7, 10, 19, 21, 24,** and **39**
(ii) cancelled: claim(s) **1-4, 8-9, 15-18,** and **22-23**
(iii) new: claim(s) **0**
(iv) replacement of one or more claims as filed, as follows: **6, 11-14, 20, 25-38,** and **40-44**
(v) the result of the division of one or more claims as filed, as follows: **0**

Dear Sir:

The Partial International Search dated **10/11/2000** designated **six** references as being relevant to patentability. In response, Claims **1-4, 8-9, 15-18,** and **22-23** as originally filed have been cancelled, and claims **6, 11-14, 20, 25-38,** and **40-44** as originally filed have been revised. The revised claims and references are addressed below seriatim.

PCT REQUEST 586.02-PCT

Original (for SUBMISSION) - printed on 18.05.2000 04:22:06 PM

0 0-1	**For receiving Office use only** International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4 0-4-1	**Form - PCT/RO/101 PCT Request** Prepared using	PCT-EASY Version 2.90 (updated 10.05.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	International Bureau of the World Intellectual Property Organization (RO/IB)
0-7	**Applicant's or agent's file reference**	586.02-PCT
I	**Title of invention**	DIFFERENTIALLY EXPRESSED GENES IN PROSTATE CANCER
II II-1 II-2 II-4 II-5 II-6 II-7 II-8 II-9 II-10	**Applicant** This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence Telephone No. Facsimile No. e-mail	 applicant and inventor all designated States SAATCIOGLU, Fahri University of Oslo Biotechnology Center of Oslo PO Box 1125 Gaustadalleen 21 N-Oslo N-1317 Blindern Norway TR NO 47 22 95 87 61 47 22 69 41 30 fahri.saatcioglu@biotek.uio.no

PCT REQUEST

586.02-PCT

Original (for **SUBMISSION**) - printed on 18.05.2000 04:22:06 PM

V	Designati n f States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT (patent and utility model) AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ (patent and utility model) DE (patent and utility model) DK (patent and utility model) DM DZ EE (patent and utility model) ES FI (patent and utility model) GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK (patent and utility model) SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	**Exclusion(s) from precautionary designations**	NONE
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	20 May 1999 (20.05.1999)
VI-1-2	Number	60/135,325
VI-1-3	Country	US

PCT REQUEST

586.02-PCT

-Original (for **SUBMISSION**) - printed on 18.05.2000 04:22:06 PM

VI-2	**Priority claim of earlier national application**		
VI-2-1	Filing date	20 May 1999 (20.05.1999)	
VI-2-2	Number	60/135,333	
VI-2-3	Country	US	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	3	-
VIII-2	Description	26	-
VIII-3	Claims	5	-
VIII-4	Abstract	1	abstract.txt
VIII-5	Drawings	6	-
VIII-7	TOTAL	41	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	5	
VIII-19	**Language of filing of the international application**	English	
IX	**Signature of applicant or agent**		
IX-1	Name (LAST, First)		
IX-2	Capacity		

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

PCT (ANNEX - FEE CALCULATION SHEET)

586.02-PCT

Original (for SUBMISSION) - printed on 18.05.2000 04:22:06 PM

(This sheet is not part of and does not count as a sheet of the international application)

0 0-1	For receiving Office use only International Application No.				
0-2	Date stamp of the receiving Office				
0-4 0-4-1	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet Prepared using	PCT-EASY Version 2.90 (updated 10.05.2000)			
0-9	Applicant's or agent's file reference	586.02-PCT			
2	Applicant	SAATCIOGLU, Fahri			
12	Calculation of prescribed fees		fee amount/multiplier	total amounts (USD)	
12-1	Transmittal fee	T	⇨	200	
12-2	Search fee	S	⇨	990	
12-3	International fee Basic fee (first 30 sheets)	b1	427		
12-4	Remaining sheets		11		
12-5	Additional amount	(X)	10		
12-6	Total additional amount	b2	110		
12-7	b1 + b2 =	B	537		
12-8	Designation fees Number of designations contained in international application		86		
12-9	Number of designation fees payable (maximum 8)		8		
12-10	Amount of designation fee	(X)	92		
12-11	Total designation fees	D	736		
12-12	PCT-EASY fee reduction	R	~~-184~~	~~1,141~~ 1,273	
12-13	Total International fee (B+D-R)	I	⇨		
12-17	TOTAL FEES PAYABLE (T+S+I+P)		⇨	~~2,331~~ 2,463	
12-19	Mode of payment	cheque			
12-20	Deposit account instructions The receiving Office:	International Bureau of the World Intellectual Property Organization (RO/IB)			
12-20-2	is hereby authorized to charge any deficiency or credit any over-payment in the total fees indicated above to my deposit account	✓ Robert DeTuro			
12-20-3	is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account	✓ Robert DeTuro			
12-21	Deposit account No.	500341			
12-22	Date	18 May 2000 (18.05.2000)			

PCT (ANNEX - FEE CALCULATION SHEET) 586.02-PCT

Original (for SUBMISSION) - printed on 18.05.2000 04:22:06 PM

12-23	Nam and signature	

VALIDATION LOG AND REMARKS

13-2-6	Validation messages Contents	Green? Priority 1. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)
		Green? Priority 2. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)
13-2-9	Validation messages Annotate	Green? The name of the person signing the request has not been indicated.

PCT

586.02-PCT

Original (for SUBMISSION) - print d on 18.05.2000 04:22:06 PM

PCT-EASY INFORMATION SHEET

(For applicant us only, DO NOT submit this sheet with th international application)

VALIDATION LOG

	Contents
Gre n?	Priority 1. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)
Gre n?	Priority 2. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)
	Annotate
Gre n?	The name of the person signing the request has not been indicated.

Before submitting the International Application, please carefully verify that:

-the information contained on printed Request form is correct;
-B x IX of the Request form has been signed;
-all elements of the international application as indicated in Box VIII of the Request form have been attached; and,
-the diskette containing the PCT-EASY zip file of the International Application has been enclosed and has been clearly labeled "PCT-EASY", with the applicant's or agent's file reference, and the first applicant's name.

ATTENTION

DO NOT modify any indications on the Request form printout. The attached PCT-EASY application has been locked. If an error or an omission is discovered at this time, you must copy the submitted application as a template and make the change or correction in a n w application (using the submitted application as a template). You may create such a template by copying the submitted application from the "Stored Forms" folder to the "New PCT Forms" folder. Open the new (.0WO) file created in the "New PCT Forms" folder, correct the errors and proceed with the submission process again.

PATENT
Attorney Docket No.: 586.02-PCT

DIFFERENTIALLY EXPRESSED GENES IN PROSTATE CANCER

This application claims the benefit of U.S. provisional application number 60/135,325 filed May 20, 1999, and U.S. provisional application number 60/135,333 filed May 20, 1999, both of which are incorporated herein by reference in their entirety.

Field of The Invention

The field of the invention is neoplastic diseases, and especially detection and therapy of prostate cancer.

Background of The Invention

Prostate cancer has become the most commonly diagnosed malignancy in males in the western world, and is the second most common cause of cancer death among men in Europe and the United States (Boring, C.C., Squires, T.S., and Tong, T. (1993). *Cancer J. Clin.* 43, 7-26; Carter, H.B., Pianpadosi, S., and Isaacs, J.T. (1990). *J. Urol.* 143, 742-746). Worse yet, in recent years the annual incidence rate of newly diagnosed prostate cancer, as well as the number of prostate cancer deaths continuously rose.

Androgens not only play a key role in the development and maintenance of the normal prostate, but also in the initiation and progression of prostate cancer (Moore, R.A. (1944). *Surgery* 16, 152-167; Huggins, C., and Johnson, M.A. (1947). *J. Am. Med. Assoc.* 135, 1146-1152). For example, androgens typically induce cell proliferation and inhibit cell death in the healthy prostate gland. Withdrawal of androgens stops proliferation of cells and induces apoptosis with concomitant involution of the prostate gland. Involution upon androgen withdrawal is generally a characteristic of a normal prostate gland as well as of a prostate tumor in the early stages of the disease, when the tumor still remains androgen dependent. Consequently, androgen withdrawal treatment is commonly used to reverse tumor growth. However, in the case of many prostate tumors, the tumor recurs after a few months or years almost invariably in an androgen insensitive state. At this point, successful therapy of prostate cancer is difficult and prognosis for survival is usually relatively low.

Almost all of the biological effects of androgens are mediated by the androgen receptor, a hormone-activated transcription factor. Even though the androgen receptor was cloned over ten years ago, the mechanisms by which the androgen receptor regulates gene expression is not well-

understood. Furthermore, only a very few of its target genes have been identified, including the prostate specific antigen (PSA), the related glandular kallikrein 2 (hKLK2), and the androgen receptor itself. Another androgen regulated protein, a secreted serine protease with prostate restricted expression, termed "prostase" was recently described by Nelson et al. (Nelson, P.S., et al. (1999) *Proc. Natl. Acad. Sci.* 96, 3114-3119). However, the biological functions of the proteins coded by the PSA, hKLK2 and prostase genes are poorly understood at present.

Adding to the difficulties in understanding the role of androgens in prostate cancer is that *in vivo* and *in vitro* model systems frequently do not closely mimic the human disease. Furthermore, close homologues of the PSA gene, the only marker for prostate cancer in human, are not known in other animal species. Moreover, *in vitro* studies are hampered due to the limited number of cell lines that have been derived from human prostate. For example, the only androgen sensitive and androgen responsive cell line that is widely used is LNCaP, characterized by cells originally derived from a lymph node metastasis of a human prostate carcinoma (Horoszewicz, J.S., Leong, S.S., Kawinski, E., Karr, J.P., Rosenthal, H., Chu, T.M., Mirand, E.A., and Murphy, G.P. (1983). *Cancer Res.* 43, 1809-1818).

In spite of numerous studies on the effects of androgens in the role of prostate cancer, relatively little is known about the molecular genetic effects of androgens in prostate cells. More detailed knowledge about androgen responsive genes and their role in signal transduction, as well as gross morphological and physiological transformations will potentially result in better diagnostic tools, and provide possible new targets for a drug-based therapy of prostate cancer. Therefore, there is still a need to provide improved methods to identify androgen responsive, differentially expressed genes in prostate cancer.

Summary of the Invention

The present invention is directed to differentially expressed genes in neoplastic cells, and particularly relates to hormone dependent genes in prostate cancer. The polynucleotides with the SEQ ID NO:1 - SEQ ID NO:7 encode an intracellular protein, and while the corresponding polypeptides SEQ ID NO:11 - SEQ ID NO:14 have an intracellular location, the corresponding expression products SEQ ID NO:8 - SEQ ID NO:10 have predominantly perinuclear, nuclear and predominantly nuclear localization within a cell, respectively.

In one aspect of the inventive subject matter, SEQ ID NO:1 - SEQ ID NO:7 are expressed in prostate cancer cells in a hormone dependent manner.

In another aspect of the inventive subject matter, a method of detecting a neoplastic cell in a system includes a step in which a predetermined amount of an RNA comprising at least one of SEQ ID NO:15 - SEQ ID NO:21 is correlated with the presence of a neoplastic cell, and the predetermined amount, or more, is subsequently detected in the system. Contemplated detection methods preferably employ a labeled probe that is detectable via fluorescence detection, luminescence detection, scintigraphy, autoradiography, or formation of a dye. Alternative preferred detection methods include addition of at least one nucleotide to the probe (*e.g.*, PCR, LCR).

In a further aspect of the inventive subject matter, a method of detecting a neoplastic cell in a system includes a step in which a predetermined amount of a polypeptide comprising at least one of SEQ ID NO:8 - SEQ ID NO:14 is correlated with the presence of a neoplastic cell, and the predetermined amount, or more, is subsequently detected in the system. Contemplated detection methods preferably employ a labeled probe that is detectable via fluorescence detection, luminescence detection, scintigraphy, autoradiography, or formation of a dye, and preferred probes include antibodies, antibody fragments, and natural and synthetic ligands of the polypeptide.

In a still further aspect of the inventive subject matter, a method of identifying differentially expressed genes in a target tissue has one step in which a target tissue-specific cDNA library is prepared by suppression subtractive hybridization, and a plurality of genes from the library is immobilized on a solid phase. Nucleic acid preparations from treated and untreated target tissue are individually hybridized with array, respectively, and hybridization patterns are compared to identify differentially expressed genes.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention, along with the accompanying drawing.

Brief Description of The Drawing

Figures 1A and 1B depict schematic nucleic acid and amino acid based multiple sequence alignments of SEQ ID NO:1 - SEQ ID NO:7 and SEQ ID NO:8 - SEQ ID NO:13, respectively.

Figure 2 is a photograph of an exemplary reverse northern blot of several clones from a cDNA library of androgen treated prostate cancer cells.

Figure 3 is a photograph of an exemplary multiple tissue northern blot of one of the isolated polynucleotides.

Figure 4 is a photograph of an agarose gel after electrophoretic separation of splicing variants of SEQ ID NO:1.

Figure 5 is a series of photomicrographs illustrating the intracellular localization of GFP-fusion proteins of polypeptides of SEQ ID NO:8 - SEQ ID NO:10.

Figure 6 is an autoradiograph of a northern blot indicating hormone dependent expression of SEQ ID NO:1 - SEQ ID NO:7.

Detailed Description

As used herein, the term "intracellular protein" refers to a protein that is expressed and retained within a cell irrespective of its subcellular localization. For example, DNA polymerase (nucleus), glyceraldehyde-3-phosphate dehydrogenase (cytoplasm), and cytochrome c oxidase (mitochondria) are all considered intracellular proteins. In contrast, the term "extracellular protein" refers to a protein that is exported from a cell. There are various mechanisms by which a protein can be exported from a cell, all of which are contemplated herein. For example, while many exported proteins have a signal sequence that allows specific export of the protein across a cell membrane, other proteins are exported through vesicles via the endoplasmatic reticulum, etc.

As also used herein, a protein has a "predominantly perinuclear localization" when a majority of the protein (*i.e.*, more than 50% of the total amount as fluorimetrically detectable by GFP-fusion) is located in a volume around the nucleus that does not exceed a volume greater than three volumes of the nucleus. A protein has a "predominantly nuclear localization" when a majority of the protein is located within the nucleus. In contrast, the term "nuclear localization"

means that substantially all of the detectable protein is located within the nucleus. Localization of a protein can be determined fluorimetrically by GFP-fusion (GFP: green fluorescence protein).

Employing a novel combined SSH-reverse northern blot procedure (first disclosed in provisional application 60/135,325 filed May 20, 1999), we isolated seven cDNAs (SEQ ID NO:1 - SEQ ID NO:7) from human prostate cancer cells. All seven cDNA molecules code for an intracellular protein, which is expressed in an androgen and glucocorticoid dependent manner. Remarkably, the sequences SEQ ID NO:1 - SEQ ID NO:7 show a high degree of homology/identity with prostase, a previously reported serine protease (Nelson, P.S., et al. (1999) *Proc. Natl. Acad. Sci.* 96, 3114-3119). However, in comparison with the prostase, all of the sequences SEQ ID NO:1 - SEQ ID NO:7 lack a 49 amino acid N-terminal portion corresponding to the first exon of the prostase. It should be especially appreciated that the first exon of the prostase not only includes structurally important amino acids, but also includes a signal peptide sequence that renders the serine protease a secreted, extracellular enzyme. Consequently, the cDNA molecules with the sequence of SEQ ID NO:1 - SEQ ID NO:7 encode intracellular proteins that are functionally and structurally different from the prostase, and are therefore independent and novel genes.

With respect to the base composition of SEQ ID NO:1 - SEQ ID NO:7, nucleotide sequences other that SEQ ID NO:1 - SEQ ID NO:7 are also contemplated and particularly include variations of SEQ ID NO:1 - SEQ ID NO:7 that include point mutations, insertions, deletions and any reasonable combination thereof, so long as alternative sequences encode an intracellular protein. Therefore, polynucleotides are contemplated that have at least 80%, preferably at least 85%, more preferably at least 90% and most preferably at least 95% identity with the sequences of SEQ IDNO:1 - SEQ IDNO:7, so long as contemplated polynucleotides encode an intracellular protein.

For example, point mutations may arise at any position of the sequence from an apurinic, apyrimidinic, or otherwise structurally impaired site within the cDNA. Alternatively, point mutations may be introduced by random or site-directed mutagenesis procedures (*e.g.*, oligonucleotide assisted or by error prone PCR). Likewise, deletions and/or insertions may be introduced into the sequences, and particularly preferred insertions comprise 5'- and/or 3'-fusions with a polynucleotide that encodes a reporter moiety or an affinity moiety. Other particularly

preferred insertions comprise a nucleic acid that further includes functional elements such as a promoter, enhancer, hormone responsive element, origin of replication, transcription and translation initiation sites, etc. It should especially be appreciated that where insertions with one or more functional elements are present, the resulting nucleic acid may be linear or circular (*e.g.*, transcription or expression cassettes, plasmids, etc.).

Still further contemplated variations include substitution of one or more atoms or chemical groups in the sequence with a radioactive atom or group. For example, where contemplated cDNAs are employed as a hybridization-specific probes, a fluorophor or enzyme (*e.g.*, β-galactosidase for generation of a dye, or luciferase for generation of luminescence) may be coupled to the sequence to identify position and/or quantity of a complementary sequence. Alternatively, where contemplated cDNA molecules are utilized for affinity isolation procedures, the cDNA may be coupled to a molecule that is known to have a high-affinity (*i.e.*, $K_d<10^{-4}mol^{-1}$) partner, such as biotin, or an oligo-histidyl tag. In another example, one or more phosphate groups may be exchanged for a radioactive phosphate group with a ^{32}P or ^{33}P isotope to assist in detection and quantification, where the radiolabeled cDNA is employed as a hybridization probe.

It is also contemplated that the polypeptides (encoded by SEQ ID NO:1 – SEQ ID NO:7) having the peptide sequence of SEQ ID NO:8 – SEQ ID NO:14 may be produced *in vivo* or *in vitro*, and may be chemically and/or enzymatically modified. Contemplated polypeptides can be isolated from prostate tissue or prostate cancer cells that may or may not be in a hormone dependent state. Alternatively, and especially where larger amounts (*i.e.*, >10mg) are desirable, recombinant production (*e.g.*, in a bacterial, yeast, insect cell, or mammalian cell system) may advantageously be employed to generate significant quantities of contemplated polypeptides.

It should further be appreciated that recombinant production not only offers a more economical strategy to produce contemplated polypeptides, but also allows specific modification in the amino acid sequence and composition to tailor particular biochemical, catalytic and physical properties. For example, where increased solubility of contemplated polypeptides is desirable, one or more hydrophobic amino acids may be replaced with hydrophilic amino acids. Alternatively, where reduced or increased catalytic activity is required, one or more amino acids may be replaced or eliminated. In still another example, fusion proteins with contemplated proteins are contemplated, in which an additional polypeptide is added to the N-terminus and/or C-terminus

of contemplated polypeptide. Particularly contemplated fusion proteins include fusions with enzymatically active fusion partners (*e.g.*, for dye formation or substrate conversion) and fluorescent fusion partners such as GFP, EGFB, BFP, etc. Therefore, sequences other than the sequences of SEQ ID NO:8 – SEQ ID NO:14 are also contemplated, so long as the polypeptides are intracellular proteins, and alternative peptide sequences may have a sequence that has a 70%, preferably 80%, more preferably 90%, and most preferably 95% homology to the sequences of SEQ ID NO:8 – SEQ ID NO:14.

With respect to chemical and enzymatic modifications of contemplated polypeptides, it is contemplated that many modifications are appropriate, including addition of mono-, and bifunctional linkers, coupling with protein- and non-protein macromolecules, and glycosylation. For example, mono- and bifunctional linkers are especially advantageous where contemplated polypeptides are immobilized to a solid support, or covalently coupled to a molecule that enhances immunogenicity of contemplated polypeptides (*e.g.*, KLH, or BSA conjugation). Alternatively, contemplated polypeptides may be coupled to antibodies or antibody fragments to allow rapid retrieval of the polypeptide from a mixture of molecules. Further contemplated couplings include covalent and non-covalent coupling of contemplated polypeptides with molecules that prolong the serum half-life and/or reduce immunogenicity such as cyclodextranes and polyethylene glycols.

In a particularly contemplated aspect of the inventive subject matter, SEQ ID NO: 15 – SEQ ID NO:21 (the corresponding mRNA of SEQ ID NO: 1 – SEQ ID NO:7) are employed in a method of detecting a neoplastic cell in a system. In one step, a predetermined quantity of an RNA comprising at least one of SEQ ID NO:15 - SEQ ID NO:21 in a cell containing system is correlated with the presence of a neoplastic cell, wherein the RNA encodes an intracellular polypeptide, and in a further step, an amount of at least the predetermined quantity of the RNA is detected in the system.

In a preferred embodiment, the system is a mammal (most preferably a human) and the neoplastic cell is a prostate cancer cell in a biopsy specimen. The total RNA is extracted from the biopsy specimen, and a real time quantitative rt-PCR employing individual reactions with primer pairs specific to each of the sequences of SEQ ID NO:15 - SEQ ID NO:21 is performed in parallel with a biopsy specimen known to be free of cancer cells. Biopsy specimens are determined to have a cancer cell, where the detected mRNA quantity of SEQ ID NO:15 - SEQ ID

NO:21 is at least 3 times higher than in the control specimen. A preferred extraction of total RNA utilizes the Quiagen BioRobot kit in conjunction with the BioRobot 9600 system, and the real time rtPCR is performed in a Perkin Elmer ABI Prism 7700.

In alternative aspects of the inventive subject matter, the method of detecting a neoplastic cell need not be limited to biopsy tissues from prostate tissue, but may employ various alternative tissues, including lymphoma tumor cells, and various solid tumor cells, so long as such tumor cells overproduce mRNA of the SEQ ID NO:15 - SEQ ID NO:21. Appropriate alternative tumor cells can readily be identified by the above described method. Likewise, the system need not be restricted to a mammal, but may also include cell-, and tissue cultures grown *in vitro*, and tumor cells and specimens from animals other than mammals.

For example, tumor cell and tissue grown *in vitro* may advantageously be utilized to investigate drug action on such cells, and the overabundance of sequences of SEQ ID NO:15 - SEQ ID NO:21 may conveniently be employed as tumor marker. Alternatively, body fluids (*e.g.*, serum, saliva, etc.) that may or may not contain tumor cells are also contemplated a suitable substrate for the method presented herein, so long as they contain to at least some extent mRNA with a sequence of SEQ ID NO:15 - SEQ ID NO:21.

With respect to the detection method it is contemplated that many methods other than quantitative real time rt-PCR are also appropriate, and particularly contemplated methods include hybridization of a probe to at least one of SEQ ID NO:15 - SEQ ID NO:21. It is especially contemplated that suitable probes are labeled, and depending on the physico-chemical nature of the probe, the detection process may include fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye. For example, for microscopic analysis of biopsy specimens, fluorescein modified sequence probes (complementary to at least one of SEQ ID NO:15 - SEQ ID NO:21) are particularly advantageous. Fluorescence quantification may then be performed utilizing a CCD-video analysis package. Similarly, luminescence may be detected with a luminometer coupled to a microscope, or where tissue pieces are submerged in a sample cuvette, luminescence may be determined in the sample fluid. It should be appreciated that labeling of oligonucleotides and hybridization of the labeled oligonucleotide is a technique that is well known in the art, and that all known methods are generally suitable for use in conjunction with methods contemplated herein. Alternatively, the amount of mRNA may also be determined by first hybridizing a probe to the mRNA and subsequently enzymatically coupling of at least

one nucleotide to the probe, and especially contemplated enzymatic additions include LCR and PCR.

In still other aspects of contemplated methods, the mRNA quantity need not necessarily be limited to at least 3 times more than in the control specimen in order to establish that the tissue has a cancer cell. For example, where the concentration of mRNA is hormone dependent, higher amounts between 3-8 fold and more may be appropriate. In contrast, where the concentration of cancer cells in the biopsy specimen is relatively low, amounts of less than 3-fold, including 1.5 to 2.9-fold and less are contemplated.

In another particularly contemplated aspect of the inventive subject matter, polypeptide of SEQ ID NO: 8 – SEQ ID NO:14 (encoded by SEQ ID NO: 1 – SEQ ID NO:7) are employed in a method of detecting a neoplastic cell in a system. In one step, a predetermined quantity of an intracellular polypeptide comprising at least one of SEQ ID NO:8 - SEQ ID NO:14 in a cell containing system is correlated with the presence of a neoplastic cell, and in a further step, an amount of at least the predetermined quantity of the RNA is then detected in the system.

In a preferred embodiment, the system is a mammal (most preferably a human) and the neoplastic cell is a prostate cancer cell or a breast cancer cell in a biopsy specimen. The biopsy specimen that is suspected to have a cancer cell is flash frozen, dissected on a microtome, and sections are mounted on microscope slides. The sections are subsequently incubated with a fluorescein labeled antibody that is directed against an epitope of at least one of the polypeptides of SEQ ID NO:8 - SEQ ID NO:14. Fluorescence is detected with a fluorescence microscope coupled to a CCD-video camera and image analysis equipment. Biopsy specimens are determined to have a cancer cell, where the fluorescence signal/quantity of one or more cells is at least 3 times higher than in the control specimen.

In alternative aspects of the inventive subject matter, the method of detecting a neoplastic cell need not be limited to biopsy tissues from prostate tissue, but may employ various alternative tissues, including lymphoma tumor cells, and various solid tumor cells, so long as such tumor cells overproduce polypeptides of the SEQ ID NO:8 - SEQ ID NO:14. Appropriate alternative tumor cells can readily be identified by the above described method. Likewise, the system need not be restricted to a mammal, but may also include cell, and tissue cultures grown *in vitro*, and tumor cells and specimens from animals other than mammals. For example, tumor cell and

tissue grown *in vitro* may advantageously be utilized to investigate drug action on such cells, and the polypeptides of SEQ ID NO:8 - SEQ ID NO:14 may conveniently be employed as a tumor marker. Alternatively, body fluids (*e.g.*, serum, saliva, etc.) that may or may not contain tumor cells are also contemplated as suitable substrates for the method presented herein, so long as they contain to at least some extent the polypeptides of SEQ ID NO:8 - SEQ ID NO:14.

With respect to detection methods, it is contemplated that many methods other than fluorescence microscopy are also appropriate, and particularly contemplated methods include specific binding of a probe to at least one of SEQ ID NO:8 - SEQ ID NO:14. It is especially contemplated that suitable probes are labeled, and depending on the physico-chemical nature of the probe, the detection process may include fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

For example, for microscopic analysis of biopsy specimens, luciferase labeled probes are particularly advantageous in conjunction with a luminescence substrate (*e.g.*, luciferin). Luminescence quantification may then be performed utilizing a CCD-camera and image analysis system. Similarly, radioactivity may be detected via autoradiographic or scintigraphic procedures on a tissue section, in a fluid or on a solid support. Where the probe is a natural or synthetic ligand of contemplated polypeptides, particularly contemplated ligands include molecules with a chemical modification that increase the affinity to the polypeptide and/or induce irreversible binding to the polypeptide. For example, transition state analogs or suicide inhibitors for a particular reaction catalyzed by the polypeptide are especially contemplated. Labeling of antibodies, antibody fragments, small molecules, and binding of the labeled entity is a technique that is well known in the art, and it is contemplated that all known methods are generally suitable for use in conjunction with methods contemplated herein. Furthermore, the probe need not be limited to a fluorescein labeled antibody, and alternative probes include antibody fragments (*e.g.*, Fab, Fab', scFab, etc.).

In still other aspects of contemplated methods, the polypeptide quantity need not necessarily be limited to at least 3 times more than the control specimen in order to establish that the tissue has a cancer cell (*e.g.*, where the control reads 100ng, three times more than the control means 300ng). For example, where the concentration of the polypeptide is hormone dependent, higher amounts between 3-8 fold and more may be appropriate. In contrast, where the concen-

tration of cancer cells in the biopsy specimen is relatively low, amounts of less than 3-fold, including 1.5 to 2.9-fold and less are contemplated.

It should further be appreciated that the polynucleotides of SEQ ID NO:1- SEQ ID NO:7 and SEQ ID NO:15 - SEQ ID NO:21 may be employed as a therapeutic modality in an antisense DNA/RNA based therapy. Anti-sense therapy, for example, could be employed to inhibit, up-, or down-regulate transcription or translation of the genes corresponding to SEQ ID NO:1- SEQ ID NO:7. It should further be appreciated that an anti-sense approach may also include regulatory sequences associated with SEQ ID NO:1- SEQ ID NO:7 such as transcription enhancers, hormone responsive elements, ribosomal- and RNA polymerase binding sites, etc., which may be located upstream or downstream of SEQ ID NO:1- SEQ ID NO:7, and may have a distance of several ten base pairs to several ten thousand base pairs.

Alternatively, the polypeptides of SEQ ID NO:8- SEQ ID NO:14 may also be employed in an antibody based therapy or a small molecule drug therapy directed towards the polypeptides of SEQ ID NO:8- SEQ ID NO:14. For example, antibody based therapy could be employed to neutralize, or remove corresponding polypeptides of SEQ ID NO:8- SEQ ID NO:14 *in-vivo*, or to interfere with one or more cellular functions of contemplated polypeptides.

Figures 1A and 1B show a schematic and an amino acid based alignment between the cDNAs of SEQ ID NO:1 - SEQ ID NO:7, in which SEQ ID NO:1 is the full-length cDNA and SEQ ID NO2 - SEQ ID NO:7 are splicing variants of SEQ ID NO:1. In the amino acid based alignment SEQ ID NO:8 is the corresponding polypeptide to SEQ ID NO:1, and SEQ ID NO:9 - SEQ ID NO:14 are the corresponding polypeptides to SEQ ID NO:2 - SEQ ID NO:6.

Examples

The following examples illustrate the isolation and cloning of the sequences of SEQ ID NO:1 - SEQ ID NO:7 form normal prostate tissue and from prostate cancer cells. SEQ ID NO:8 - SEQ ID NO:14, and SEQ ID NO15 - SEQ ID NO:21 are computer generated transcriptions and translations of SEQ ID NO1 - SEQ ID NO:7, respectively.

The following examples also illustrate a general method of identifying differentially expressed genes in a target tissue, in which in one step a target tissue-specific cDNA library is provided that has a plurality of tissue-specific genes obtained by suppression subtractive

hybridization. In a subsequent step, a predetermined quantity of tissue-specific genes is immobilized on a solid phase to form a tissue-specific cDNA array, and a first nucleic acid preparation is hybridized to a first tissue-specific cDNA array to create a first hybridization pattern, wherein the first preparation is prepared from the target tissue without previously exposing the target tissue to a compound. In a further step, a second nucleic acid preparation is hybridized to a second tissue-specific cDNA array to create a second hybridization pattern, wherein the second preparation is prepared from the target tissue after previously exposing the target tissue to a compound. In yet a further step, the first and the second hybridization pattern are then compared to identify differentially expressed genes. This general method is especially contemplated where the compound comprises a hormone, or various other suitable ligands.

Suppression Subtraction of Prostate Specific Genes

cDNA derived from poly(A)+ RNA of 10 different normal human tissues were subtracted against normal human prostate cDNA using suppression subtraction hybridization (SSH) (Diatchenko, L., Lau, Y.-F- C., Campbell, A.P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E.D., Siebert, P.D. (1996). *Proc. Natl. Acad. Sci.* USA 93, 6025-6030), and the resulting cDNA fragments were cloned into an appropriate vector. SSH was performed as described (Clontech PCR-Select Cloning Kit) using prostate poly(A)+ RNA against a pool of poly(A)+ RNA obtained from ten normal human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, spleen, thymus, and ovary). Upon secondary PCR amplification (12 cycles), reactions were extracted with phenol/chloroform and DNA was precipitated with EtOH.

The pellet was washed once with 70% EtOH. After drying, the DNA pellet was dissolved in 0.2xTE or dH_2O and cut with RsaI in a 20 ul reaction for 2 hrs at 37C to excise adaptors. After digestion, reactions were run on a 1.5 % agarose gel, with molecular size markers on one side, at 5 V/cm, 40 min. The adopter bands are excised and discarded, and cDNA bands were cut out and purified (QAIEX gel DNA purification kit) after running the gel backwards to concentrate the cDNA.

The purified DNA was subcloned into EcoRV-cut, dephosphorylated pZERO vector from Invitrogen. DH10B electrocompetent cells (>10^{10} efficiency) were transformed with a 1/5 dilution of 1µl of the ligation mix.

Colonies were picked and the presence of cDNA inserts confirmed by PCR with T7 and SP6 primers directly from the colonies. 10% of reactions were run on a 1.5% agarose gel to visualize amplified products. The colonies with inserts were grown and glycerol stocks (15%) were prepared and stored at –80C.

Reverse Northern Blot and Sequence Analyses

Clones from the SSH library were amplified by PCR and spotted on nylon filters in 96-well format to generate two identical blots for each set of 92 clones (the remaining four spots were used for positive and negative controls). For probe preparation, the androgen-responsive prostate cancer cell line LNCaP (Horoszewicz, J.S., Leong, S.S., Kawinski, E., Karr, J.P., Rosenthal, H., Chu, T.M., Mirand, E.A., and Murphy, G.P. (1983). *Cancer Res.* 43, 1809-1818) was employed that was either untreated [the (-) probe] or treated with the synthetic androgen R1881 for 24 hours [the (+) probe]. Poly(A)+ RNA was isolated from these cells and was used to make the ^{32}P-labeled probes. After hybridization with the (-) and (+) probes, clones showing differential hybridization were selected for further analysis (*i.e.*, confirmation by a secondary reverse northern blot, and northern blotting).

Reverse northern screening on the cDNA clones were done essentially as described elsewhere (Hedrick, S.M., Cohen, D.I., Neilson, E.A., Davis, M.M. (1984) *Nature* 308, 149-153; Sakaguchi N, Berger CN, Melchers F (1986). *EMBO J* 5: 2139-2147). DNA (approximately 400 ng) from PCR amplification in step 6 was diluted in 200 µl of 0.4M NaOH, 10 mM EDTA and mixed well by pipetting. After incubation at 95°C for 5-10 min, the tubes were chilled on ice. Denatured DNA was blotted on two separate pieces of Zeta Probe GT+ membrane (Bio-Rad) using a dot-blot apparatus (Bio-Rad). Positive [Prostate specific antigen (PSA) cDNA] and negative [glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA] controls are included on each blot in duplicate. Membranes were rinsed with 2XSSC, air dried, and then baked at 80°C for 30 min. A typical example of a reverse northern analysis is shown in **Figure 2.** In each blot pair, PSA and G3PDH are included as positive and negative controls, respectively. It should be noted that there was substantial increase in PSA hybridization in the (+) blot (probe prepared from cells that have been stimulated by androgens) compared with the (-) blot (probe prepared from untreated cells), whereas there was no significant change in hybridization of G3PDH between the two blots. Arrowheads indicate differentially expressed clones.

As a control to verify the prostate specific nature of isolated sequences, positive clones were tested in a standard northern blot against RNA preparations of multiple non-prostate tissues and a typical blot is shown in **Figure 3**. Lanes 1-10, and 12-16 are RNA preparations from non-prostate tissues, lane 11 is a RNA preparation from prostate, lane 12 is a RNA preparation from testis.

Probes: The probes were random-prime radiolabeled using standard laboratory procedures. Unincorporated nucleotides were removed using prespun G25 columns (Bio-Rad), and specific activity was typically over $5x10^8$cpm/μg.

Hybridization: 25 ml Hybridization mix (7% SDS, 0.5 M $NaHPO_4$, 1mM EDTA) at 65°C is prewarmed, and 12.5 ml were used for prehybridization of each membrane for 5-10 min at 65 °C. Probes were heat denatured at 95°C for 3-5 min and transferred to the prehybridization mix at 65°C. Hybridization was done at 65°C overnight.

Washing: Wash solution I (2xSSC and 1% SDS) and wash solution II (0.1xSSC and 0.5% SDS) were prewarmed. Membranes were washed once with Solution I and then Solution II for 30 min at 65°C, covered with plastic wrap and exposed to phoshorimager screen.

Selection: Clones showing differential expression between the (-) and (+) blots were picked. A secondary round of reverse northern analysis is performed for confirmation by spotting each clone in duplicate on each blot. To confirm hormone dependence, a time course of R1881 induction of LNCaP cells as well as the CWR22 xenograft model upon androgen ablation (Wainstein, M. A., He, F., Robinson, D., Kung, H. J., Schwartz, S., Giaconia, J. M., Edgehouse, N. L., Pretlow, T. P., Bodner, D. R., Kursh, E. D., and Pretlow, T.G. (1994). *Cancer Res.* 54, 6049-6052) and the androgen-independent CWR22R relapsed xenograft (Nagabhushan, M., Miller, C. M., Pretlow, T. P., Giaconia, J. M., Edgehouse, N. L., Schwartz, S., Kung, H. J., de Vere White, R. W., Gumerlock, P. H., Resnick, M. I., Amini, S. B. , and Pretlow, T. G. (1996). *Cancer Res. 56,* 3042-6) was used.

Sequence analysis: Sequence analysis was performed by the dideoxy chain termination methods using an ABI automated sequencer. It should be appreciated that many more androgen responsive, differentially expressed genes can be identified and isolated using the cloning strategy outlined above, including genes expressed during various growth and developmental phases

of a diseased prostate, and genes expressed as a result of a drug regimen. Moreover, it is contemplated that not only differentially expressed prostate cancer genes can be identified and isolated, but also genes involved in other diseased states of human prostate, including benign prostate hyperplasia, etc.

Isolation of Splice Variants (SEQ ID NO: 2 - SEQ ID NO: 7)

Poly(A)$^+$ RNA was isolated from LNCaP cells treated with R1881 (a synthetic androgen) and from androgen dependent prostate cancer xenograft CWR22 grown in nude mice in the presence of androgens. cDNA was prepared and subjected to PCR using SEQ ID NO:1 specific primers and a primer pair designed to amplify the previously published prostase. The respective 5'-primers were located around the translation start site, while the 3'-primer for all reactions was located around the stop codon. Reaction products were loaded onto an agarose gel and separated as shown in **Figure 4**. Lane 1 is a marker, lane 2 is positive control with SEQ ID NO:1 as template. Lanes 3-5 are PCR products from CWR22 cells with SEQ ID NO:1 specific primers, while lanes 6-8 are PCR products from CWR22 cells with prostase specific 5'-primer. Lanes 9-11 are PCR products from LNCaP cells with SEQ ID NO:1 specific primers, and lanes 12-14 are PCR products from LNCaP cells with prostase specific primers. Lane 15 is marker.

Only reactions with SEQ ID NO:1 specific primers yielded detectable PCR products, with a major band at 680bp (SEQ ID NO:1), and two additional bands at about 500bp (SEQ ID NO:2) and 750bp (SEQ ID NO:3). When primers for 5'-RACE analysis were employed, four additional PCR products were obtained, corresponding to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively. All bands were sequenced to confirm their identity. SEQ ID NO:7 was obtained both as a 3'-RACE product as well as a distinct clone from the PSL library.

Localization of Intracellular Proteins encoded by SEQ ID NO: 1 - SEQ ID NO: 3

To gain insight into the subcellular location of the polypeptides SEQ ID NO:8 - SEQ ID NO:10 (encoded by SEQ ID NO:1 - SEQ ID NO:3), C-terminal fusion constructs with GFP were produced in COS7 cells. The cells were fixed, stained with DAPI and visualized by phase contrast or fluorescence microscopy and representative images are shown in **Figure 5**. Photographs in lane A depict a GFP fusion protein with SEQ ID NO:9, the photographs in lane B depict a GFP fusion protein with SEQ ID NO:10, the photographs in lane C depict a GFP fusion protein with SEQ ID NO:8, and the photographs in lane D depict a GFP protein as a control. The poly-

peptide of SEQ ID NO: 8 displayed strong granular fluorescence predominantly around the nucleus, while the polypeptide of SEQ ID NO:9 and SEQ ID NO:10 showed exclusively nuclear and predominantly nuclear localization, respectively. Due to the lack of an identifiable leader sequence that would indicate an export of the polypeptides of SEQ ID NO:11 - SEQ ID NO:14, it is contemplated that the sequences SEQ ID NO:11 - SEQ ID NO:14 are also intracellular proteins.

Regulation of Expression by multiple Hormones

Untreated LNCaP cells and hormone treated LNCaP cells were employed to determine the hormone dependence of expression of SEQ ID NO:1. Treatment was as follows: Testosterone (T) at 10^{-8}M, dihydrotestosterone (DHT) at 10^{-8}M, estradiol (E2) at 10^{-8}M, progesterone (P) at 10^{-8}M, dexamethasone (Dex) at 10^{-7}M, 1, 25-dihydroxy-vitamin D3 (VitD3) at 10^{-8}M, and triiodothyronine (T3) at 10^{-7}M. The total RNA of the treated cells was isolated and used in a northern blot analysis with radiolabeled SEQ ID NO:1 as probe. **Figure 6** shows the results of an autoradiograph of a northern blot as described above. 18S-RNA is shown as control for RNA integrity and loading. The relative induction of SEQ ID NO:1 is indicated at the bottom of the lanes as determined by phosphorimager analysis. It is contemplated that SEQ ID NO:2 - SEQ ID NO:7 are splice variants of SEQ ID NO:1, and consequently it is contemplated that the expression of all of SEQ ID NO:1 - SEQ ID NO:6 is hormone dependent, and particularly contemplated hormones include androgens, progesterones, estrogens and glucocorticoids.

Thus, specific embodiments and applications of methods and applications of differentially expressed genes in prostate cancer cells have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

Canceled

SEQUENCE LISTING

SEQ ID NO:1

atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60

gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120

caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180

agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240

gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300

gtttctggct ggggtctgct ggcgaacggc agaatgccta ccgtgctgca gtgcgtgaac 360

gtgtcggtgg tgtctgagga ggtctgcagt aagctctatg acccgctgta ccaccccagc 420

atgttctgcg ccggcggagg gcaagaccag aaggactcct gcaacggtga ctctgggggg 480

cccctgatct gcaacgggta cttgcagggc cttgtgtctt tcggaaaagc cccgtgtggc 540

caagttggcg tgccaggtgt ctacaccaac ctctgcaaat tcactgagtg gatagagaaa 600

accgtccagg ccagttaa 618

SEQ ID NO:2

atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60

gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120

caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180

agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240

gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300

gtttctggct ggggtctgct ggcgaacggg tgactctggg gggcccctga tctgcaacgg 360

gtacttgcag ggccttgtgt ctttcggaaa agccccgtgt ggccaagttg gcgtgccagg 420

tgtctacacc aacctctgca aattcactga gtggatagag aaaaccgtcc aggccagtta 480

a 481

SEQ ID NO:3

atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60

gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120

caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180

agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240

gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300

gtttctggct ggggtctgct ggcgaacggt gagctcacgg gtgtgtgtct gccctcttca 360

aggaggtcct ctgcccagtc gcgggggctg acccagagct ctgcgtccca ggcagaatgc 420

ctaccgtgct gcagtgcgtg aacgtgtcgg tggtgtctga ggaggtctgc agtaagctct 480

atgacccgct gtaccacccc agcatgttct gcgccggcgg agggcaagac cagaaggact 540

cctgcaacgg tgactctggg ggggccctga tctgcaacgg gtacttgcag ggccttgtgt 600

ctttcggaaa agccccgtgt tggccaagtt ggcgtgccag gtgtctacac caacctctgc 660

aaattcactg agtggataga gaaaaccgtc caggccagtt aa 702

SEQ ID NO:4

ggaatgagcc tggatccggg gagcccagag ggaagggctg ggaggcggga atcttgcttc 60

ggaaggactc agagagtcct gacttgaaat ctcagcccag tgctgagtct ctagtgaact 120

aagctcctac accatcgggc tgggcctgca cagtcttgag gccgaccaag agccagggag 180

ccagatggtg gaggccagcc tctccgtacg gcacccagag tacaacagac ccttgctcgc 240

taacgacctc atgctcatca agttggacga atccgtgtcc gagtctgaca ccatccggag 300

catcagcatt gcttcgcagt gccctaccgc ggggaactct tgcctcgttt ctggctgggg 360

tctgctggcg aacggtgaac tcacgggtgt gtgtctgccc tcttcaagga ggtcctctgc 420

ccagtcgcgg gggctgaccc agagctctgc gtcccaggca gaatgcctac cgtgctgcag 480

tgcgtgaacg tgtcggtggt gtctgaggag gtctgcagta agctctatga cccgctgtac 540

caccccagca tgttctgcgc cggcggaggg caagaccaga aggactcctg caacggtgac 600

tctgggggc ccctgatctg caacgggtac ttgcagggcc ttgtgtcttt cggaaaagcc 660

ccgtgtggcc aagttggcgt gccaggtgtc tacaccaacc tctgcaaatt cactgagtgg 720

atagagaaaa ccgtccaggc cagttaactc tggggactgg gaacccatga aattgacccc 780

caaatacatc ctgcggaagg aattcaggaa tatctgatcc cagcccctcc tccc 834

SEQ ID NO:5

ggaatgagcc tggatccggg gagcccagag ggaagggctg ggaggcggga atcttgcttc 60

ggaaggactc agagagccct gacttgaaat ctcagcccag tgctgagtct ctagtgaact 120

aagctcctac accatcgggc tgggcctgca cagtcttgag gccgaccaag agccagggag 180

ccagatggtg gaggccagcc tctccgtacg gcacccagag tacaacagac ccttgctcgc 240

taacgacctc atgctcatca agttggacga atccgtgtcc gagtctgaca ccatccggag 300

catcagcatt gcttcgcagt gccctaccgc ggggaactct tgcctcgttt ctggctgggg 360

tctgctggcg aacggcagaa tgcctaccgt gctgcagtgc gtgaacgtgt cggtggtgtc 420

tgaggaggtc tgcagtaagc 440

SEQ ID NO:6

ggctctggga ggaggacgga atgagcctgg atccggggag cccagaggga agggctggga 60

ggcgggaatc ttgcttcgga aggactcaga gagccctgac ttgaaatctc agcccagtgc 120

tgagtctcta gtgaactaag ctcctacacc atcgggctgg gcctgcacag tcttgaggcc 180

gaccaagagc cagggagcca gatggtggag gccagcctct ccgtacggca cccagagtac 240

aacagaccct tgctcgctaa cgacctcatg ctcatcaagt tggacgaatc cgtgtccgag 300

tctgacacca tccggagcat cagcattgct tcgcagtgcc ctaccgcggg gaactcttgc 360

ctcgtttctg gctggggtct gctggcgaac ggcagaatgc ctaccgtgct gcagtgcgtg 420

aacgtgtcgg tggtgtctga ggaggtctgc agtaagc 457

SEQ ID NO:7

accaccccag catgttctgc gccggcggag agcaagacca gaaggactcc tgcaacgtga 60

gagaggggaa aggggagggc aggcgactca gggaagggtg gagaaggggg agacagagac 120

acacagggcc gcatggcgag atgcagagat ggagagacac acagggagac agtgacaact 180

agagagagaa actgagagaa acagggaaat aaacacagga ataaagagaa gcaaaggaag 240

agagaaacag aaacagacat gggggaggca gaaacacaca cacatagaaa tgcagctgac 300

cttccaacag catggggcct gagggcggtg acctccaccc aacagaaaat cctcttataa 360

cttttgactc cccaaaaaac ctgactagaa atagcctact gttgacgggg gagccttacc 420

aataacataa atagtcgatt tatgcatacg ttttatgcat tcatgatata cctttgttgg 480

aatttttga tatttctaag ctacacagtt cgtctgtgaa ttttttaaa ttgttgcaac 540

tctcctaaaa ttttttctaa tgtgtttatt gaaaaaaatc caagtataag tggacttgtg 600

cagttcaaac cagggttgtt caagggtcaa ctgtgt 636

SEQ ID NO:8

auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60

gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120

caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180

agacccuugc ucgcuaacga ccucaugcuc aucaaguugg acgaauccgu guccgagucu 240

gacaccaucc ggagcaucag cauugcuucg cagugcccua ccgcggggaa cucuugccuc 300

guuucuggcu ggggucugcu ggcgaacggc agaaugccua ccgugcugca gugcgugaac 360

gugucggugg ugucugagga ggucugcagu aagcucuaug acccgcugua ccaccccagc 420

auguucugcg ccggcggagg gcaagaccag aaggacuccu gcaacgguga cucugggggg 480

ccccugaucu gcaacgggua cuugcagggc cuugugucuu ucggaaaagc cccguguggc 540

caaguuggcg ugccaggugu cuacaccaac cucugcaaau ucacugagug gauagagaaa 600

accguccagg ccaguuaa 618

SEQ ID NO:9

auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60

gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120

caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180

agacccuugc ucgcuaacga ccucaugcuc aucaaguugg acgaauccgu guccgagucu 240

gacaccaucc ggagcaucag cauugcuucg cagugcccua ccgcggggaa cucuugccuc 300

guuucuggcu ggggucugcu ggcgaacggg ugacucuggg gggccccuga ucugcaacgg 360

guacuugcag ggccuugguc uuucggaaaa gccccgugug gccaaguugg cgugccaggu 420

gucuacacca accucugcaa auucacugag uggauagaga aaaccgucca ggccaguuaa 480

SEQ ID NO:10

auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60

gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120

caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180

agacccuugc ucgcuaacga ccucagcuca ucaaguugga cgaauccgug uccgagucug 240

acaccauccg gagcaucagc auugcuucgc agugcccuac cgcggggaac ucuugccucg 300

uuucuggcug gggucugcug gcgaacggug agcucacggg ugugugucug cccucuucaa 360

ggaggcccuc ugcccagucg cgggggcuga cccagagcuc ugcgucccag gcagaaugcc 420

uaccgugcug cagugcguga acgugucggu ggugucugag gaggucugca guaagcucua 480

ugacccgcug uaccacccca gcauguucug cgccggcgga gggcaagacc agaaggacuc 540

cugcaacggu gacucugggg gggcccugau cugcaacggg uacuugcagg gccuuguguc 600

uuucggaaaa gccccguguu ggccaaguug gcgugccagg ugucuacacc aaccucugca 660

aauucacuga guggauagag aaaaccgucc aggccaguua a 701

SEQ ID NO:11

ggaaugagcc uggauccggg gagcccagag ggaagggcug ggaggcggga aucuugcuuc 60

ggaaggacuc agagaguccg acuugaaauc ucagcccagu gcugagucuc uagugaacua 120

agcuccuaca ccaucgggcu gggccugcac agucuugagg ccgaccaaga gccagggagc 180

cagaggugga ggccagccuc uccguacggc acccagagua caacagaccc uugcucgcua 240

acgaccucau gcucaucaag uuggacgaau ccguguccga gucugacacc auccggagca 300

ucagcauugc uucgcagugc ccuaccgcgg ggaacucuug ccucguuucu ggcuggggguc 360

ugcuggcgaa cgggaacuca cgggugugug ucugcccucu ucaaggaggu ccucugccca 420

gucgcggggg cugacccaga gcucugcguc ccaggcagaa gccuaccgug cugcagugcg 480

ugaacguguc gguggugucu gaggaggucu gcaguaagcu cuaugacccg cuguaccacc 540

ccagcauguu cugcgccggc ggagggcaag accagaagga cuccugcaac ggugacucug 600

gggggccccu gaucugcaac ggguacuugc agggccuugu gucuuucgga aaagccccgu 660

guggccaagu uggcgugcca ggugucuaca ccaaccucug caaauucacu gaguggauag 720

agaaaaccgu ccaggccagu uaacucuggg gacugggaac ccaugaaauu gacccccaaa 780

uacauccugc ggaaggaauu caggaauauc ugaucccagc cccuccuccc 830

SEQ ID NO:12

ggaaugagcc uggauccggg gagcccagag ggaagggcgg gaggcgggaa ucuugcuucg 60

gaaggacuca gagagcccug acuugaaauc ucagcccagu gcugagucuc uagugaacua 120

agcuccuaca ccacgggcug ggccugcaca gucuugaggc cgaccaagag ccagggagcc 180

agauggugga ggccagccuc uccguacggc acccagagua caacagaccc uugcucgcua 240

acgaccucau gcucaucaag uuggacgaau ccguguccga gucugacacc auccggagca 300

ucagcauugc uucgcagugc ccuaccgcgg ggaacucuug ccucguuucu ggcugggguc 360

ugcuggcgaa cggcagaaug ccuaccgugc ugcagugcgu gaacgugucg guggugucug 420

aggaggucug caguaagc 438

SEQ ID NO:13

gcucugggag gaggacggaa ugagccugga uccggggagc ccagagggaa gggcugggag 60

gcgggaaucu ugcuucggaa ggacucagag agcccugacu ugaaaucuca gcccagugcu 120

gagucucuag ugaacuaagc uccuacacca ucgggcuggg ccugcacagu cuugaggccg 180

accaagagcc agggagccag augguggagg ccagccucuc cguacggcac ccagaguaca 240

acagacccuu gcucgcuaac gaccucaugc caucaaguug gacgaauccg uguccgaguc 300

ugacaccauc cggagcauca gcauugcuuc gcagugcccu accgcgggga acucuugccu 360

cguuucuggc uggggucugc uggcgaacgg cagaaugccu accgugcugc agugcgugaa 420

cgugucggug gugucugagg aggucugcag uaagc 455

SEQ ID NO:14

accaccccag cauguucugc gccggcggag agcaagacca gaaggacucc ugcaacguga 60

gagaggggaa aggggagggc aggcgacuca gggaagggug gagaaggggg agacagagac 120

acacagggcc gcauggcgag augcagagau ggagagacac acagggagac agugacaacu 180

agagagagaa acugagagaa acagggaaau aaacacagga auaaagagaa gcaaaggaag 240

agagaaacag aaacagacau gggggaggca gaaacacaca cacauagaaa ugcagcugac 300

cuuccaacag caugggccu gagggcggug accuccaccc aacagaaaau ccucuuauaa 360

cuuuugacuc cccaaaaaac cugacuagaa auagccuacu guugacgggg gagccuuacc 420

aauaacauaa auagucgauu uaugcauacg uuuuaugcau ucaugauaua ccuuuguugg 480

aauuuuuuga uauuucuaag cuacacaguu cgucugugaa uuuuuuuaaa uuguugcaac 540

ucuccuaaaa uuuuuucuaa uguguuuauu gaaaaaaauc caaguaaagu ggacuugugc 600

aguucaaacc aggguuguuc aagggucaac ugugu 635

SEQ ID NO:15

Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp

Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu

Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met
Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val
Cys Ser Lys Leu Tyr Asp Pro Leu Tyr His Pro Ser Met Phe Cys Ala
Gly Gly Gly Gln Asp Gln Lys Asp Ser Cys Asn Gly Asp Ser Gly Gly
Pro Leu Ile Cys Asn Gly Tyr Leu Gln Gly Leu Val Ser Phe Gly Lys
Ala Pro Cys Gly Gln Val Gly Val Pro Gly Val Tyr Thr Asn Leu Cys
Lys Phe Thr Glu Trp Ile Glu Lys Thr Val Gln Ala Ser

SEQ ID NO:16

Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp
Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu
Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly

SEQ ID NO:17

Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp
Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu
Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Glu Leu
Thr Gly Val Cys Leu Pro Ser Ser Arg Arg Ser Ser Ala Gln Ser Arg

Gly Leu Thr Gln Ser Ser Ala Ser Gln Ala Glu Cys Leu Pro Cys Cys Ser Ala

SEQ ID NO:18

Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Glu Leu Thr Gly Val Cys Leu Pro Ser Ser Arg Arg Ser Ser Ala Gln Ser Arg Gly Leu Thr Gln Ser Ser Ala Ser Gln Ala Glu Cys Leu Pro Cys Cys Ser Ala

SEQ ID NO: 19

Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val Cys Ser Lys

SEQ ID NO:20

Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val Cys Ser Lys

SEQ ID NO:21

Ala Ile Ser Ser Gln Val Phe Trp Gly Val Lys Ser Tyr Lys Arg Ile

Phe Cys Trp Val Glu Val Thr Ala Leu Arg Pro His Ala Val Gly Arg

Ser Ala Ala Phe Leu Cys Val Cys Val Ser Ala Ser Pro Met Ser Val

Ser Val Ser Leu Phe Leu Cys Phe Ser Leu Phe Leu Cys Leu Phe Pro

Cys Phe Ser Gln Phe Leu Ser Leu Val Val Thr Val Ser Leu Cys Val

Ser Pro Ser Leu His Leu Ala Met Arg Pro Cys Val Ser Leu Ser Pro

Pro Ser Pro Pro Phe Pro Glu Ser Pro Ala Leu Pro Phe Pro Leu Ser

His Val Ala Gly Val Leu Leu Val Leu Leu Ser Ala Gly Ala Glu His

Ala Gly Val

CLAIMS

What is claimed is:

1. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:1, wherein the nucleotide sequence encodes an intracellular protein.

2. The polynucleotide of claim 2 wherein the intracellular protein has a predominantly peri-nuclear localization in a cell.

3. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:2, wherein the nucleotide sequence encodes an intracellular protein.

4. The polynucleotide of claim 3 wherein the intracellular protein has a nuclear localization in a cell.

5. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:3, wherein the nucleotide sequence encodes an intracellular protein.

6. The polynucleotide of claim 5 wherein the intracellular protein has a predominantly nuclear localization in a cell.

7. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:4, wherein the nucleotide sequence encodes an intracellular protein.

8. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:5, wherein the nucleotide sequence encodes an intracellular protein.

9. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:6, wherein the nucleotide sequence encodes an intracellular protein.

10. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:7, wherein the nucleotide sequence encodes an intracellular protein.

11. The polynucleotide of any one of claim 2, 4, or 6 wherein the polynucleotide is expressed *in vivo* in a prostate cancer cell.

12. The polynucleotide of any one of claims 2, 4, or 6 wherein the expression of the polynucleotide is dependent on at least one of an androgen, a progesterone, an estrogen, and a glucocorticoid.

13. A polynucleotide having at least 90% identity to at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

14. A polynucleotide having at least 95% identity to at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

15. A polypeptide comprising the amino acid sequence of SEQ ID NO:8, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

16. The polypeptide of claim 15 wherein the polypeptide has a predominantly perinuclear localization in a cell.

17. A polypeptide comprising the amino acid sequence of SEQ ID NO:9, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

18. The polypeptide of claim 17 wherein the polypeptide has a nuclear localization in a cell.

19. A polypeptide comprising the amino acid sequence of SEQ ID NO:10, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

20. The polypeptide of claim 19 wherein the polypeptide has a predominantly nuclear localization in a cell.

21. A polypeptide comprising the amino acid sequence of SEQ ID NO:11, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

22. A polypeptide comprising the amino acid sequence of SEQ ID NO:12, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

23. A polypeptide comprising the amino acid sequence of SEQ ID NO:13, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

24. A polypeptide comprising the amino acid sequence of SEQ ID NO:14, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

25. A polypeptide having at least 90% homology to at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

26. A polypeptide having at least 95% homology to at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

27. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an RNA comprising at least one of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 in a cell containing system with a presence of a neoplastic cell, wherein the RNA encodes an intracellular polypeptide; and

detecting at least the predetermined quantity of the RNA in the system.

28. The method of claim 27 wherein the neoplastic cell is a prostate cancer cell.

29. The method of claim 27 wherein the system is a mammal.

30. The method of claim 27 wherein the step of detecting includes hybridization of a probe to at least one of the SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

31. The method of claim 30 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

32. The method of claim 30 wherein at least one nucleotide is enzymatically coupled to the probe while the probe is hybridized to the at least one of the SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

33. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an intracellular polypeptide comprising at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14 in a cell containing system with a presence of a neoplastic cell; and

detecting at least the predetermined quantity of the intracellular polypeptide in the system.

34. The method of claim 33 wherein the neoplastic cell is a prostate cancer cell or a breast cancer cell.

35. The method of claim 33 wherein the system is a mammal.

36. The method of claim 33 wherein the step of detecting includes specifically binding of a probe to the polypeptide.

37. The method of claim 36 wherein the probe is selected from the group consisting of an antibody, an antibody fragment, a natural ligand of the polypeptide, and a synthetic ligand of the polypeptide.

38. The method of claim 36 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

39. A method of identifying differentially expressed genes in a target tissue, comprising:

providing a target tissue-specific cDNA library having a plurality of tissue-specific genes, wherein the tissue-specific genes are obtained by suppression subtractive hybridization;

immobilizing a predetermined quantity of tissue-specific genes on a solid phase to form a tissue-specific cDNA array;

hybridizing a first nucleic acid preparation to a first tissue-specific cDNA array to create a first hybridization pattern, wherein the first preparation is prepared from the target tissue without previously exposing the target tissue to a compound;

hybridizing a second nucleic acid preparation to a second tissue-specific cDNA array to create a second hybridization pattern, wherein the second preparation is prepared from the target tissue after previously exposing the target tissue to a compound; and

comparing the first and the second hybridization pattern to identify differentially expressed genes.

40. The method of claim 39 wherein the target tissue comprises prostate tissue.

41. The method of claim 40 wherein the prostate tissue comprises prostate cancer cells.

42. The method of claim 39 wherein the solid phase comprises a membrane.

43. The method of claim 39 wherein the compound comprises a hormone.

44. The method of claim 39 wherein at least one of the first and second nucleic acid preparations is radiolabeled and wherein the step of comparing comprises phosphorimaging.

ABSTRACT

SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 encode an intracellular protein that is expressed in prostate epithelial cells in a hormone dependent manner. Encoded proteins SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 have predominantly perinuclear, nuclear and predominantly nuclear location localization within a cell, respectively. In contemplated methods of detecting a neoplastic cell in a system, a predetermined amount of at least one of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, or at least one of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 is correlated with the presence of a neoplastic cell and detected within the system employing specific binding of a labeled probe. In a method of identifying differentially expressed genes, a tissue specific array of cDNA prepared by suppression subtractive hybridization is arranged on a solid phase. Two nucleic acid preparations are individually hybridized with the array, wherein the first and second nucleic acid preparations are prepared from treated and untreated target tissue. A comparison of the hybridization patterns reveals differentially expressed genes.

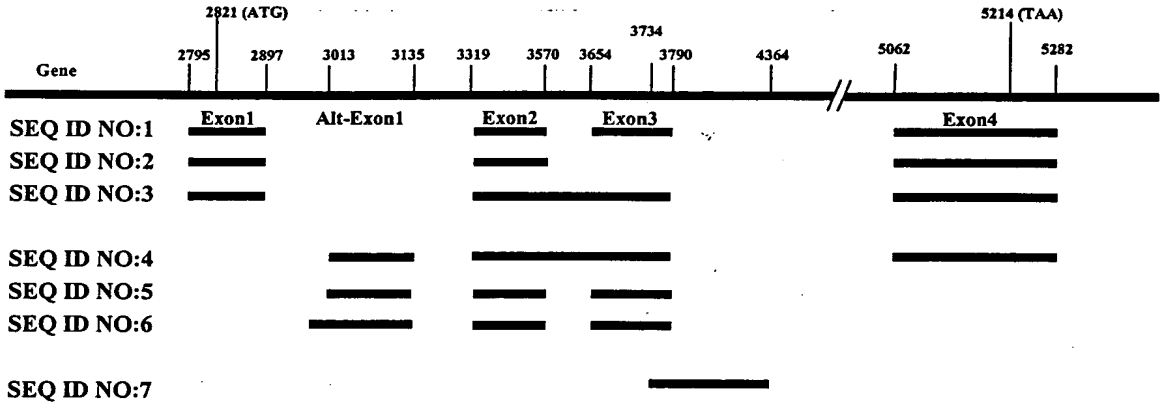


Figure 1A

```
                  *         20        *         40        *         60        *         80        *        100        *
SEQ ID NO:8 : MENELFCSGVLVHPQWVLSAAHCFQNSYTIGLGLHSLEADQEPGSQMVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG : 110
SEQ ID NO:9 : MENELFCSGVLVHPQWVLSAAHCFQNSYTIGLGLHSLEADQEPGSQMVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG : 110
SEQ ID NO:10: MENELFCSGVLVHPQWVLSAAHCFQNSYTIGLGLHSLEADQEPGSQMVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG : 110
SEQ ID NO:11: ----------------------------------------MVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG :  64
SEQ ID NO:12: ----------------------------------------MVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG :  64
SEQ ID NO:13: ----------------------------------------MVEASLSVRHPEYNRPLLANDLMLIKLDESVSESDTIRSISIASQCPTAGNSCLVSGWGLLANG :  64

                       120       *       140       *       160       *       180       *       200       *
SEQ ID NO:8 : RMP-----------TVLQCVNVSVVSEEVCSKLYDPLYHPSMFCAGGGQDQKDSCNGDSGGPLICNGYLQGLVSFGKAPCGQVGVPGVYTNLCKFTEWIEKTVQAS : 205
SEQ ID NO:9 : ------------------------------------------------------------------------------------------------------------ :   -
SEQ ID NO:10: ELTGVCLPSSRRSSAQSRGLTQSSASQAESLPCCSA------------------------------------------------------------------------ : 146
SEQ ID NO:11: ELTGVCLPSSRRSSAQSRGLTQSSASQAESLPCCSA------------------------------------------------------------------------ : 100
SEQ ID NO:12: RMP-----------TVLQCVNVSVVSEEVCSK------------------------------------------------------------------------ :  85
SEQ ID NO:13: RMP-----------TVLQCVNVSVVSEEVCSK------------------------------------------------------------------------ :  85
```

Figure 1B

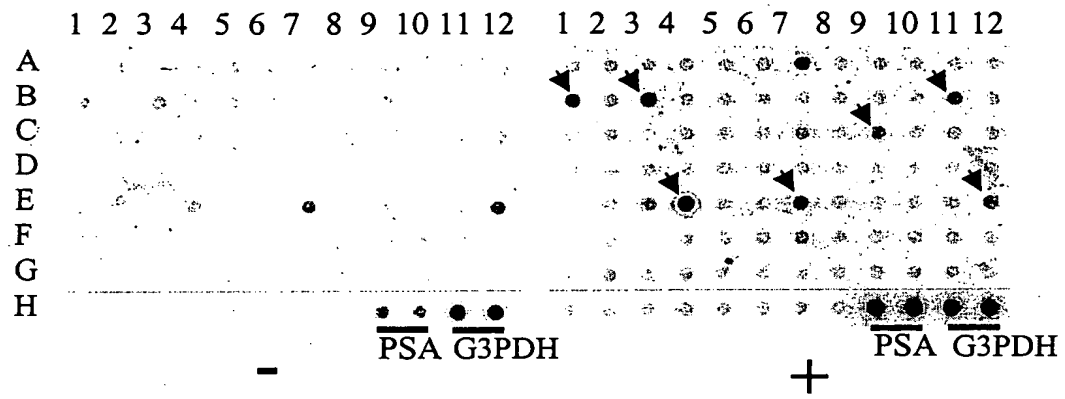


Figure 2

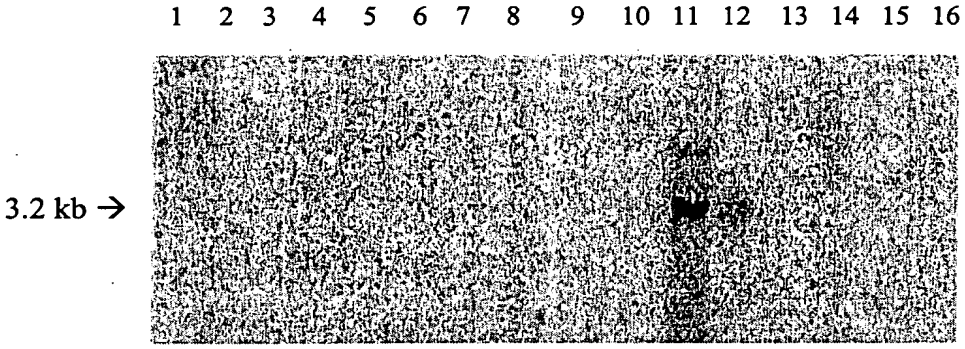


Figure 3

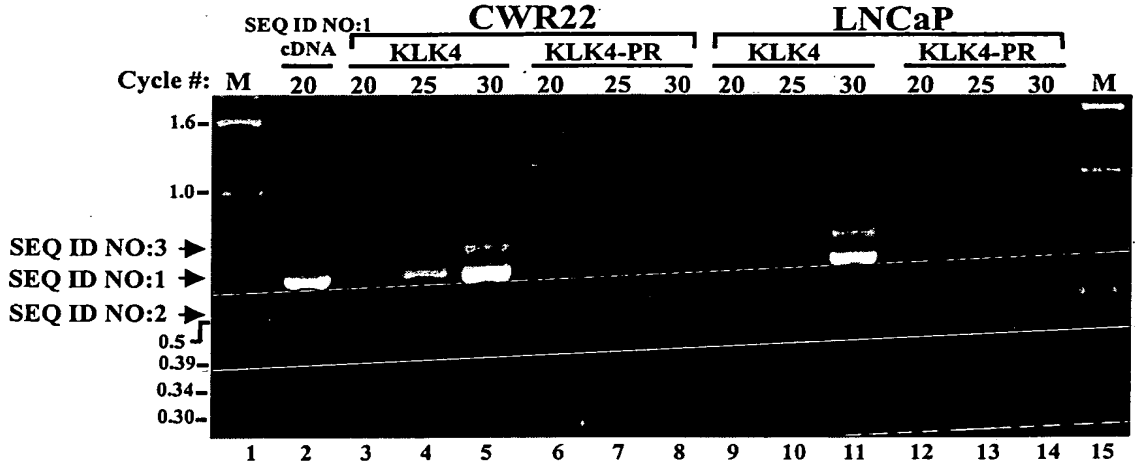


Figure 4

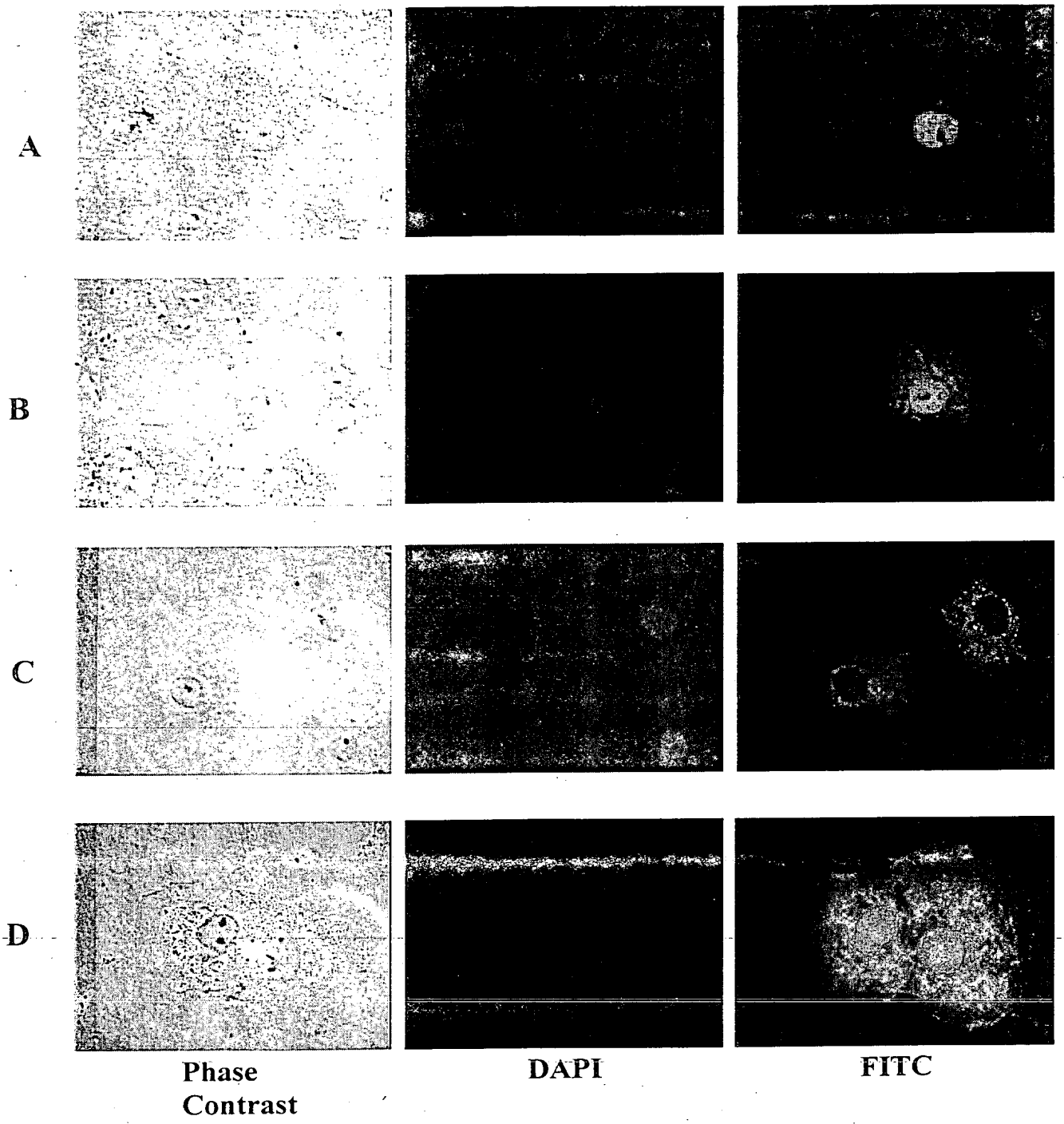


Figure 5

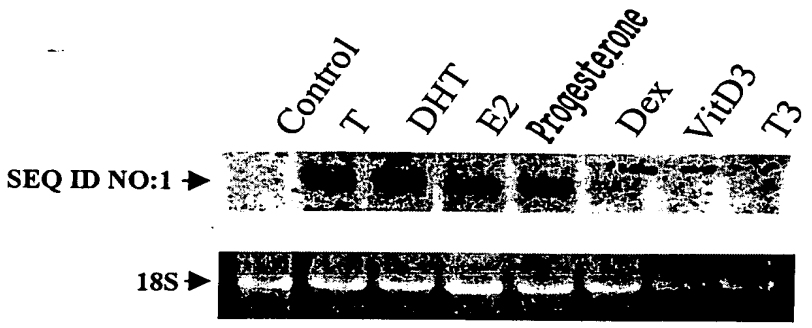


Figure 6

SEQUENCE LISTING

<110> Saatcioglu, Fahri

<120> Differentially Expressed Genes in Prostate Cancer

<130> Sequences for 586.02-PCT

<140>
<141>

<150> 60/135,325
<151> 1999-05-20

<150> 60/135,333
<151> 1999-05-20

<160> 21

<170> PatentIn Ver. 2.0

<210> 1
<211> 618
<212> DNA
<213> Homo sapiens

<400> 1
atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60
gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120
caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180
agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240
gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300
gtttctggct ggggtctgct ggcgaacggc agaatgccta ccgtgctgca gtgcgtgaac 360
gtgtcggtgg tgtctgagga ggtctgcagt aagctctatg acccgctgta ccaccccagc 420
atgttctgcg ccggcggagg gcaagaccag aaggactcct gcaacggtga ctctgggggg 480
cccctgatct gcaacgggta cttgcagggc cttgtgtctt tcggaaaagc cccgtgtggc 540
caagttggcg tgccaggtgt ctacaccaac ctctgcaaat tcactgagtg gatagagaaa 600
accgtccagg ccagttaa 618

<210> 2
<211> 481
<212> DNA
<213> Homo sapiens

<400> 2
atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60
gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120
caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180

agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240
gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300
gtttctggct ggggtctgct ggcgaacggg tgactctggg gggcccctga tctgcaacgg 360
gtacttgcag ggccttgtgt ctttcggaaa agccccgtgt ggccaagttg gcgtgccagg 420
tgtctacacc aacctctgca aattcactga gtggatagag aaaaccgtcc aggccagtta 480
a 481

<210> 3
<211> 702
<212> DNA
<213> Homo sapiens

<400> 3
atggaaaacg aattgttctg ctcgggcgtc ctggtgcatc cgcagtgggt gctgtcagcc 60
gcacactgtt tccagaactc ctacaccatc gggctgggcc tgcacagtct tgaggccgac 120
caagagccag ggagccagat ggtggaggcc agcctctccg tacggcaccc agagtacaac 180
agacccttgc tcgctaacga cctcatgctc atcaagttgg acgaatccgt gtccgagtct 240
gacaccatcc ggagcatcag cattgcttcg cagtgcccta ccgcggggaa ctcttgcctc 300
gtttctggct ggggtctgct ggcgaacggt gagctcacgg gtgtgtgtct gccctcttca 360
aggaggtcct ctgcccagtc gcgggggctg acccagagct ctgcgtccca ggcagaatgc 420
ctaccgtgct gcagtgcgtg aacgtgtcgg tggtgtctga ggaggtctgc agtaagctct 480
atgacccgct gtaccacccc agcatgttct gcgccggcgg agggcaagac cagaaggact 540
cctgcaacgg tgactctggg ggggccctga tctgcaacgg gtacttgcag ggccttgtgt 600
ctttcggaaa agccccgtgt tggccaagtt ggcgtgccag gtgtctacac caacctctgc 660
aaattcactg agtggataga gaaaaccgtc caggccagtt aa 702

<210> 4
<211> 834
<212> DNA
<213> Homo sapiens

<400> 4
ggaatgagcc tggatccggg gagcccagag ggaagggctg ggaggcggga atcttgcttc 60
ggaaggactc agagagtcct gacttgaaat ctcagcccag tgctgagtct ctagtgaact 120
aagctcctac accatcgggc tgggcctgca cagtcttgag gccgaccaag agccagggag 180
ccagatggtg gaggccagcc tctccgtacg gcacccagag tacaacagac ccttgctcgc 240
taacgacctc atgctcatca agttggacga atccgtgtcc gagtctgaca ccatccggag 300
catcagcatt gcttcgcagt gccctaccgc ggggaactct tgcctcgttt ctggctgggg 360
tctgctggcg aacggtgaac tcacgggtgt gtgtctgccc tcttcaagga ggtcctctgc 420
ccagtcgcgg gggctgaccc agagctctgc gtcccaggca gaatgcctac cgtgctgcag 480
tgcgtgaacg tgtcggtggt gtctgaggag gtctgcagta agctctatga cccgctgtac 540
caccccagca tgttctgcgc cggcggaggg caagaccaga aggactcctg caacggtgac 600
tctgggggggc ccctgatctg caacgggtac ttgcagggcc ttgtgtcttt cggaaaagcc 660
ccgtgtggcc aagttggcgt gccaggtgtc tacaccaacc tctgcaaatt cactgagtgg 720
atagagaaaa ccgtccaggc cagttaactc tggggactgg gaacccatga aattgacccc 780
caaatacatc ctgcggaagg aattcaggaa tatctgatcc cagcccctcc tccc 834

<210> 5

<211> 440
<212> DNA
<213> Homo sapiens

<400> 5
ggaatgagcc tggatccggg gagcccagag ggaagggctg ggaggcggga atcttgcttc 60
ggaaggactc agagagccct gacttgaaat ctcagcccag tgctgagtct ctagtgaact 120
aagctcctac accatcgggc tgggcctgca cagtcttgag gccgaccaag agccagggag 180
ccagatggtg gaggccagcc tctccgtacg gcacccagag tacaacagac ccttgctcgc 240
taacgacctc atgctcatca agttggacga atccgtgtcc gagtctgaca ccatccggag 300
catcagcatt gcttcgcagt gccctaccgc ggggaactct tgcctcgttt ctggctgggg 360
tctgctggcg aacggcagaa tgcctaccgt gctgcagtgc gtgaacgtgt cggtggtgtc 420
tgaggaggtc tgcagtaagc 440

<210> 6
<211> 457
<212> DNA
<213> Homo sapiens

<400> 6
ggctctggga ggaggacgga atgagcctgg atccggggag cccagaggga agggctggga 60
ggcgggaatc ttgcttcgga aggactcaga gagccctgac ttgaaatctc agcccagtgc 120
tgagtctcta gtgaactaag ctcctacacc atcgggctgg gcctgcacag tcttgaggcc 180
gaccaagagc cagggagcca gatggtggag gccagcctct ccgtacggca cccagagtac 240
aacagaccct tgctcgctaa cgacctcatg ctcatcaagt tggacgaatc cgtgtccgag 300
tctgacacca tccggagcat cagcattgct tcgcagtgcc ctaccgcggg gaactcttgc 360
ctcgtttctg gctggggtct gctggcgaac ggcagaatgc ctaccgtgct gcagtgcgtg 420
aacgtgtcgg tggtgtctga ggaggtctgc agtaagc 457

<210> 7
<211> 636
<212> DNA
<213> Homo sapiens

<400> 7
accaccccag catgttctgc gccggcggag agcaagacca gaaggactcc tgcaacgtga 60
gagaggggaa aggggagggc aggcgactca gggaagggtg gagaaggggg agacagagac 120
acacagggcc gcatggcgag atgcagagat ggagagacac acagggagac agtgacaact 180
agagagagaa actgagagaa acagggaaat aaacacagga ataaagagaa gcaaaggaag 240
agagaaacag aaacagacat gggggaggca gaaacacaca cacatagaaa tgcagctgac 300
cttccaacag catggggcct gagggcggtg acctccaccc aacagaaaat cctcttataa 360
cttttgactc cccaaaaaac ctgactagaa atagcctact gttgacgggg gagccttacc 420
aataacataa atagtcgatt tatgcatacg ttttatgcat tcatgatata cctttgttgg 480
aatttttga tatttctaag ctacacagtt cgtctgtgaa ttttttaaa ttgttgcaac 540
tctcctaaaa ttttttctaa tgtgtttatt gaaaaaaatc caagtataag tggacttgtg 600
cagttcaaac cagggttgtt caagggtcaa ctgtgt 636

<210> 8

<211> 618
<212> RNA
<213> Homo sapiens

<400> 8
auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60
gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120
caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180
agacccuugc ucgcuaacga ccucaugcuc aucaaguugg acgaauccgu guccgagucu 240
gacaccaucc ggagcaucag cauugcuucg cagugcccua ccgcggggaa cucuugccuc 300
guuucuggcu ggggucugcu ggcgaacggc agaaugccua ccgugcugca gugcgugaac 360
gugucggugg ugucugagga ggucugcagu aagcucuaug acccgcugua ccacccccagc 420
auguucugcg ccggcggagg gcaagaccag aaggacuccu gcaacgguga cucuggggggg 480
ccccugaucu gcaacgggua cuugcagggc cuugugucuu ucggaaaagc cccguguggc 540
caaguuggcg ugccaggugu cuacaccaac cucugcaaau ucacugagug gauagagaaa 600
accguccagg ccaguuaa 618

<210> 9
<211> 480
<212> RNA
<213> Homo sapiens

<400> 9
auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60
gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120
caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180
agacccuugc ucgcuaacga ccucaugcuc aucaaguugg acgaauccgu guccgagucu 240
gacaccaucc ggagcaucag cauugcuucg cagugcccua ccgcggggaa cucuugccuc 300
guuucuggcu ggggucugcu ggcgaacggg ugacucuggg gggccccuga ucugcaacgg 360
guacuugcag ggccuugguc uuucggaaaa gccccgugug gccaaguugg cgugccaggu 420
gucuacacca accucugcaa auucacugag uggauagaga aaaccgucca ggccaguuaa 480

<210> 10
<211> 701
<212> RNA
<213> Homo sapiens

<400> 10
auggaaaacg aauuguucug cucgggcguc cuggugcauc cgcagugggu gcugucagcc 60
gcacacuguu uccagaacuc cuacaccauc gggcugggcc ugcacagucu ugaggccgac 120
caagagccag ggagccagau gguggaggcc agccucuccg uacggcaccc agaguacaac 180
agacccuugc ucgcuaacga ccucagcuca ucaaguugga cgaauccgug uccgagucug 240
acaccauccg gagcaucagc auugcuucgc agugcccuac cgcggggaac ucuugccucg 300
uuucuggcug gggucugcug gcgaacggug agcucacggg ugugugucug cccucuucaa 360
ggagguccuc ugcccagucg cgggggcuga cccagagcuc ugcgucccag gcagaaugcc 420
uaccgugcug cagugcguga acgugucggu ggugucugag gaggucugca guaagcucua 480
ugacccgcug uaccacccca gcauguucug cgccggcgga gggcaagacc agaaggacuc 540
cugcaacggu gacucugggg gggcccugau cugcaacggg uacuugcagg gccuuguguc 600

uuucggaaaa gccccguguu ggccaaguug gcgugccagg ugucuacacc aaccucugca 660
aauucacuga guggauagag aaaaccgucc aggccaguua a 701

<210> 11
<211> 830
<212> RNA
<213> Homo sapiens

<400> 11
ggaaugagcc uggauccggg gagcccagag ggaagggcug ggaggcggga aucuugcuuc 60
ggaaggacuc agagaguccg acuugaaauc ucagcccagu gcugagucuc uagugaacua 120
agcuccuaca ccaucgggcu gggccugcac agucuugagg ccgaccaaga gccagggagc 180
cagaggugga ggccagccuc uccguacggc acccagagua caacagaccc uugcucgcua 240
acgaccucau gcucaucaag uuggacgaau ccguguccga gucugacacc auccggagca 300
ucagcauugc uucgcagugc ccuaccgcgg ggaacucuug ccucguuucu ggcugggguc 360
ugcuggcgaa cgggaacuca cgggugugug ucugcccucu ucaaggaggu ccucugccca 420
gucgcggggg cugacccaga gcucugcguc ccaggcagaa gccuaccgug cugcagugcg 480
ugaacguguc ggugggucu gaggaggucu gcaguaagcu cuaugacccg cuguaccacc 540
ccagcauguu cugcgccggc ggagggcaag accagaagga cuccugcaac ggugacucug 600
gggggccccu gaucugcaac ggguacuugc agggccuugu gucuuucgga aaagccccgu 660
guggccaagu uggcgugcca ggugucuaca ccaaccucug caaauucacu gaguggauag 720
agaaaaccgu ccaggccagu uaacucuggg gacugggaac ccaugaaauu gacccccaaa 780
uacauccugc ggaaggaauu caggaauauc ugaucccagc cccuccuccc 830

<210> 12
<211> 438
<212> RNA
<213> Homo sapiens

<400> 12
ggaaugagcc uggauccggg gagcccagag ggaagggcgg gaggcgggaa ucuugcuucg 60
gaaggacuca gagagcccug acuugaaauc ucagcccagu gcugagucuc uagugaacua 120
agcuccuaca ccacgggcug ggccugcaca gucuugaggc cgaccaagag ccagggagcc 180
agauggugga ggccagccuc uccguacggc acccagagua caacagaccc uugcucgcua 240
acgaccucau gcucaucaag uuggacgaau ccguguccga gucugacacc auccggagca 300
ucagcauugc uucgcagugc ccuaccgcgg ggaacucuug ccucguuucu ggcugggguc 360
ugcuggcgaa cggcagaaug ccuaccgugc ugcagugcgu gaacgugucg guggugucug 420
aggaggucug caguaagc 438

<210> 13
<211> 455
<212> RNA
<213> Homo sapiens

<400> 13
gcucugggag gaggacggaa ugagccugga uccggggagc ccagagggaa gggcugggag 60
gcgggaaucu ugcuucggaa ggacucagag agcccugacu ugaaaucuca gcccagugcu 120
gagucucuag ugaacuaagc uccuacacca ucgggcuggg ccugcacagu cuugaggccg 180

accaagagcc agggagccag augguggagg ccagccucuc cguacggcac ccagaguaca 240
acagacccuu gcucgcuaac gaccucaugc caucaaguug gacgaauccg uguccgaguc 300
ugacaccauc cggagcauca gcauugcuuc gcagugcccu accgcgggga acucuugccu 360
cguuucuggc uggggucugc uggcgaacgg cagaaugccu accgugcugc agugcgugaa 420
cgugucggug gugucugagg aggucugcag uaagc 455

<210> 14
<211> 635
<212> RNA
<213> Homo sapiens

<400> 14
accaccccag cauguucugc gccggcggag agcaagacca gaaggacucc ugcaacguga 60
gagaggggaa aggggagggc aggcgacuca gggaagggug gagaaggggg agacagagac 120
acacagggcc gcauggcgag augcagagau ggagagacac acagggagac agugacaacu 180
agagagagaa acugagagaa acagggaaau aaacacagga auaaagagaa gcaaaggaag 240
agagaaacag aaacagacau gggggaggca gaaacacaca cacauagaaa ugcagcugac 300
cuuccaacag caugggggccu gagggcggug accuccaccc aacagaaaau ccucuuauaa 360
cuuuugacuc cccaaaaaac cugacuagaa auagccuacu guugacgggg gagccuuacc 420
aauaacauaa auagucgauu uaugcauacg uuuuaugcau ucaugauaua ccuuuguugg 480
aauuuuuuga uauuucuaag cuacacaguu cgucugugaa uuuuuuuaaa uuguugcaac 540
ucuccuaaaa uuuuuucuaa uguguuuauu gaaaaaaauc caaguaaagu ggacuugugc 600
aguucaaacc aggguuguuc aagggucaac ugugu 635

<210> 15
<211> 205
<212> PRT
<213> Homo sapiens

<400> 15
Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp
1 5 10 15

Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu
20 25 30

Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
35 40 45

Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
50 55 60

Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
65 70 75 80

Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
85 90 95

Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Arg Met
100 105 110

Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu Glu Val
115 120 125

Cys Ser Lys Leu Tyr Asp Pro Leu Tyr His Pro Ser Met Phe Cys Ala
130 135 140

Gly Gly Gly Gln Asp Gln Lys Asp Ser Cys Asn Gly Asp Ser Gly Gly
145 150 155 160

Pro Leu Ile Cys Asn Gly Tyr Leu Gln Gly Leu Val Ser Phe Gly Lys
165 170 175

Ala Pro Cys Gly Gln Val Gly Val Pro Gly Val Tyr Thr Asn Leu Cys
180 185 190

Lys Phe Thr Glu Trp Ile Glu Lys Thr Val Gln Ala Ser
195 200 205

<210> 16
<211> 110
<212> PRT
<213> Homo sapiens

<400> 16
Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp
1 5 10 15

Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu
20 25 30

Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
35 40 45

Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
50 55 60

Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
65 70 75 80

Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
85 90 95

Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly
100 105 110

<210> 17
<211> 146
<212> PRT
<213> Homo sapiens

<400> 17
Met Glu Asn Glu Leu Phe Cys Ser Gly Val Leu Val His Pro Gln Trp
1 5 10 15

Val Leu Ser Ala Ala His Cys Phe Gln Asn Ser Tyr Thr Ile Gly Leu
20 25 30

Gly Leu His Ser Leu Glu Ala Asp Gln Glu Pro Gly Ser Gln Met Val
35 40 45

Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro Leu Leu
50 55 60

Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser Glu Ser
65 70 75 80

Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr Ala Gly
85 90 95

Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly Glu Leu
100 105 110

Thr Gly Val Cys Leu Pro Ser Ser Arg Arg Ser Ser Ala Gln Ser Arg
115 120 125

Gly Leu Thr Gln Ser Ser Ala Ser Gln Ala Glu Cys Leu Pro Cys Cys
130 135 140

Ser Ala
145

<210> 18
<211> 100
<212> PRT
<213> Homo sapiens

<400> 18
Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro
1 5 10 15

Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser
20 25 30

Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr
35 40 45

Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly
50 55 60

Glu Leu Thr Gly Val Cys Leu Pro Ser Ser Arg Arg Ser Ser Ala Gln
65 70 75 80

Ser Arg Gly Leu Thr Gln Ser Ser Ala Ser Gln Ala Glu Cys Leu Pro
85 90 95

Cys Cys Ser Ala
100

<210> 19
<211> 85
<212> PRT
<213> Homo sapiens

<400> 19
Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro
1 5 10 15

Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser
20 25 30

Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr
35 40 45

Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly
50 55 60

Arg Met Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu
65 70 75 80

Glu Val Cys Ser Lys
85

<210> 20
<211> 85
<212> PRT
<213> Homo sapiens

<400> 20
Met Val Glu Ala Ser Leu Ser Val Arg His Pro Glu Tyr Asn Arg Pro
1 5 10 15

Leu Leu Ala Asn Asp Leu Met Leu Ile Lys Leu Asp Glu Ser Val Ser
20 25 30

Glu Ser Asp Thr Ile Arg Ser Ile Ser Ile Ala Ser Gln Cys Pro Thr
35 40 45

Ala Gly Asn Ser Cys Leu Val Ser Gly Trp Gly Leu Leu Ala Asn Gly
50 55 60

Arg Met Pro Thr Val Leu Gln Cys Val Asn Val Ser Val Val Ser Glu
65 70 75 80

Glu Val Cys Ser Lys
85

<210> 21
<211> 131
<212> PRT
<213> Homo sapiens

<400> 21
Ala Ile Ser Ser Gln Val Phe Trp Gly Val Lys Ser Tyr Lys Arg Ile
1 5 10 15

Phe Cys Trp Val Glu Val Thr Ala Leu Arg Pro His Ala Val Gly Arg
20 25 30

Ser Ala Ala Phe Leu Cys Val Cys Val Ser Ala Ser Pro Met Ser Val
35 40 45

Ser Val Ser Leu Phe Leu Cys Phe Ser Leu Phe Leu Cys Leu Phe Pro
50 55 60

Cys Phe Ser Gln Phe Leu Ser Leu Val Val Thr Val Ser Leu Cys Val
65 70 75 80

Ser Pro Ser Leu His Leu Ala Met Arg Pro Cys Val Ser Leu Ser Pro
85 90 95

Pro Ser Pro Pro Phe Pro Glu Ser Pro Ala Leu Pro Phe Pro Leu Ser
100 105 110

His Val Ala Gly Val Leu Leu Val Leu Leu Ser Ala Gly Ala Glu His
115 120 125

Ala Gly Val
130

CLAIMS

What is claimed is:

1. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:1, wherein the nucleotide sequence encodes an intracellular protein.

2. The polynucleotide of claim 2 wherein the intracellular protein has a predominantly peri-nuclear localization in a cell.

3. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:2, wherein the nucleotide sequence encodes an intracellular protein.

4. The polynucleotide of claim 3 wherein the intracellular protein has a nuclear localization in a cell.

5. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:3, wherein the nucleotide sequence encodes an intracellular protein.

6. The polynucleotide of claim 5 wherein the intracellular protein has a predominantly nuclear localization in a cell.

7. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:4, wherein the nucleotide sequence encodes an intracellular protein.

8. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:5, wherein the nucleotide sequence encodes an intracellular protein.

9. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:6, wherein the nucleotide sequence encodes an intracellular protein.

10. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:7, wherein the nucleotide sequence encodes an intracellular protein.

11. The polynucleotide of any one of claim 2, 4, or 6 wherein the polynucleotide is expressed *in vivo* in a prostate cancer cell.

12. The polynucleotide of any one of claims 2, 4, or 6 wherein the expression of the polynucleotide is dependent on at least one of an androgen, a progesterone, an estrogen, and a glucocorticoid.

13. A polynucleotide having at least 90% identity to at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

14. A polynucleotide having at least 95% identity to at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

15. A polypeptide comprising the amino acid sequence of SEQ ID NO:8, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

16. The polypeptide of claim 15 wherein the polypeptide has a predominantly perinuclear localization in a cell.

17. A polypeptide comprising the amino acid sequence of SEQ ID NO:9, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

18. The polypeptide of claim 17 wherein the polypeptide has a nuclear localization in a cell.

19. A polypeptide comprising the amino acid sequence of SEQ ID NO:10, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

20. The polypeptide of claim 19 wherein the polypeptide has a predominantly nuclear localization in a cell.

21. A polypeptide comprising the amino acid sequence of SEQ ID NO:11, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

22. A polypeptide comprising the amino acid sequence of SEQ ID NO:12, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

23. A polypeptide comprising the amino acid sequence of SEQ ID NO:13, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

24. A polypeptide comprising the amino acid sequence of SEQ ID NO:14, wherein the amino acid sequence is expressed *in vivo* in a prostate cancer cell.

25. A polypeptide having at least 90% homology to at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

26. A polypeptide having at least 95% homology to at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

27. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an RNA comprising at least one of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 in a cell containing system with a presence of a neoplastic cell, wherein the RNA encodes an intracellular polypeptide; and

detecting at least the predetermined quantity of the RNA in the system.

28. The method of claim 27 wherein the neoplastic cell is a prostate cancer cell.

29. The method of claim 27 wherein the system is a mammal.

30. The method of claim 27 wherein the step of detecting includes hybridization of a probe to at least one of the SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

31. The method of claim 30 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography; and formation of a dye.

32. The method of claim 30 wherein at least one nucleotide is enzymatically coupled to the probe while the probe is hybridized to the at least one of the SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

33. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an intracellular polypeptide comprising at least one of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14 in a cell containing system with a presence of a neoplastic cell; and

detecting at least the predetermined quantity of the intracellular polypeptide in the system.

34. The method of claim 33 wherein the neoplastic cell is a prostate cancer cell or a breast cancer cell.

35. The method of claim 33 wherein the system is a mammal.

36. The method of claim 33 wherein the step of detecting includes specifically binding of a probe to the polypeptide.

37. The method of claim 36 wherein the probe is selected from the group consisting of an antibody, an antibody fragment, a natural ligand of the polypeptide, and a synthetic ligand of the polypeptide.

38. The method of claim 36 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

39. A method of identifying differentially expressed genes in a target tissue, comprising:

providing a target tissue-specific cDNA library having a plurality of tissue-specific genes, wherein the tissue-specific genes are obtained by suppression subtractive hybridization;

immobilizing a predetermined quantity of tissue-specific genes on a solid phase to form a tissue-specific cDNA array;

hybridizing a first nucleic acid preparation to a first tissue-specific cDNA array to create a first hybridization pattern, wherein the first preparation is prepared from the target tissue without previously exposing the target tissue to a compound;

hybridizing a second nucleic acid preparation to a second tissue-specific cDNA array to create a second hybridization pattern, wherein the second preparation is prepared from the target tissue after previously exposing the target tissue to a compound; and

comparing the first and the second hybridization pattern to identify differentially expressed genes.

40. The method of claim 39 wherein the target tissue comprises prostate tissue.

41. The method of claim 40 wherein the prostate tissue comprises prostate cancer cells.

42. The method of claim 39 wherein the solid phase comprises a membrane.

43. The method of claim 39 wherein the compound comprises a hormone.

44. The method of claim 39 wherein at least one of the first and second nucleic acid preparations is radiolabeled and wherein the step of comparing comprises phosphorimaging.

Revised Claims (Numbering as originally filed)

6. The polynucleotide of claim [5] 1 wherein the intracellular protein has a predominantly nuclear localization in a cell.

11. The polynucleotide of any one of claim [2, 4, or 6] ~~1, 3, or 4~~ wherein the polynucleotide is expressed *in vivo* in a prostate cancer cell.

12. The polynucleotide of any one of claims [2, 4, or 6] ~~1, 3, or 4~~ wherein the expression of the polynucleotide is dependent on at least one of an androgen, a progesterone, an estrogen, and a glucocorticoid.

13. A polynucleotide having at least 90% identity to at least one of [SEQ ID NO:1, SEQ ID NO:2,] SEQ ID NO:3, SEQ ID NO:4, [SEQ ID NO:5, SEQ ID NO:6,] and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

14. A polynucleotide having at least 95% identity to at least one of [SEQ ID NO:1, SEQ ID NO:2,] SEQ ID NO:3, SEQ ID NO:4, [SEQ ID NO:5, SEQ ID NO:6,] and SEQ ID NO:7, wherein the polynucleotide encodes an intracellular protein.

20. The polypeptide of claim [19] 9 wherein the polypeptide has a predominantly nuclear localization in a cell.

25. A polypeptide having at least 90% homology to at least one of [SEQ ID NO:8, SEQ ID NO:9,] SEQ ID NO:10, SEQ ID NO:11, [SEQ ID NO:12, SEQ ID NO:13,] and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

26. A polypeptide having at least 95% homology to at least one of [SEQ ID NO:8, SEQ ID NO:9,] SEQ ID NO:10, SEQ ID NO:11, [SEQ ID NO:12, SEQ ID NO:13,] and SEQ ID NO:14, wherein the polypeptide is an intracellular protein.

27. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an RNA comprising at least one of [SEQ ID NO:15, SEQ ID NO:16,] SEQ ID NO:17, SEQ ID NO:18, [SEQ ID NO:19, SEQ ID NO:20,] and SEQ ID NO:21 in a cell containing system with a presence of a neoplastic cell, wherein the RNA encodes an intracellular polypeptide; and

detecting at least the predetermined quantity of the RNA in the system.

28. The method of claim [27] 15 wherein the neoplastic cell is a prostate cancer cell.

29. The method of claim [27] 15 wherein the system is a mammal.

30. The method of claim [27] 15 wherein the step of detecting includes hybridization of a probe to at least one of the [SEQ ID NO:15, SEQ ID NO:16,] SEQ ID NO:17, SEQ ID NO:18, [SEQ ID NO:19, SEQ ID NO:20,] and SEQ ID NO:21.

31. The method of claim [30] 18 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

32. The method of claim [30] 18 wherein at least one nucleotide is enzymatically coupled to the probe while the probe is hybridized to the at least one of the [SEQ ID NO:15, SEQ ID NO:16,] SEQ ID NO:17, SEQ ID NO:18, [SEQ ID NO:19, SEQ ID NO:20,] and SEQ ID NO:21.

33. A method of detecting a neoplastic cell, comprising:

correlating a predetermined quantity of an intracellular polypeptide comprising at least one of [SEQ ID NO:8, SEQ ID NO:9,] SEQ ID NO:10, SEQ ID NO:11, [SEQ ID NO:12, SEQ ID NO:13,] and SEQ ID NO:14 in a cell containing system with a presence of a neoplastic cell; and

detecting at least the predetermined quantity of the intracellular polypeptide in the system.

34. The method of claim [33] 21 wherein the neoplastic cell is a prostate cancer cell or a breast cancer cell.

35. The method of claim [33] 21 wherein the system is a mammal.

36. The method of claim [33] 21 wherein the step of detecting includes specifically binding of a probe to the polypeptide.

37. The method of claim [36] 24 wherein the probe is selected from the group consisting of an antibody, an antibody fragment, a natural ligand of the polypeptide, and a synthetic ligand of the polypeptide.

38. The method of claim [36] 24 wherein the probe carries a label that is detected by a process selected from the group consisting of fluorescence detection, luminescence detection, scintigraphy, autoradiography, and formation of a dye.

40. The method of claim [39] 27 wherein the target tissue comprises prostate tissue.

41. The method of claim [40] 28 wherein the prostate tissue comprises prostate cancer cells.

42. The method of claim [39] 27 wherein the solid phase comprises a membrane.

43. The method of claim [39] 27 wherein the compound comprises a hormone.

44. The method of claim [39] 27 wherein at least one of the first and second nucleic acid preparations is radiolabeled and wherein the step of comparing comprises phosphorimaging.

Sequence Numbering

On page 20, change "SEQ ID NO: 8" to – SEQ ID NO: 15--.

On page 21, change "SEQ ID NO: 9" to – SEQ ID NO: 16--.

On page 21, change "SEQ ID NO: 10" to – SEQ ID NO: 17--.

On page 22, change "SEQ ID NO: 11" to – SEQ ID NO: 18--.

On page 22, change "SEQ ID NO: 12" to – SEQ ID NO: 19--.

On page 22, change "SEQ ID NO: 13" to – SEQ ID NO: 20--.

On page 23, change "SEQ ID NO: 14" to – SEQ ID NO: 21--.

On page 23, change "SEQ ID NO: 15" to – SEQ ID NO: 8--.

On page 24, change "SEQ ID NO: 16" to – SEQ ID NO: 9--.

On page 24, change "SEQ ID NO: 17" to – SEQ ID NO: 10--.

On page 24, change "SEQ ID NO: 18" to – SEQ ID NO: 11--.

On page 25, change "SEQ ID NO: 19" to – SEQ ID NO: 12--.

On page 25, change "SEQ ID NO: 20" to – SEQ ID NO: 13--.

On page 25, change "SEQ ID NO: 21" to – SEQ ID NO: 14--.

Consequently, the sequences of SEQ ID NO: 1-7 are DNA sequences, the sequences of SEQ ID NO: 8-14 are the polypeptide sequences corresponding to SEQ ID NO: 1-7, respectively, and the sequences of SEQ ID NO: 15-21 are RNA sequences corresponding to SEQ ID NO: 1-7, respectively.

Preliminary Comments

SEQ ID NO:1-6 appear to be splicing variants of a parent nucleotide sequence. While SEQ ID NO: 2, 5, and 6 are a subset of SEQ ID NO: 1, SEQ ID NO: 3 and 4 have additional sequence portions not found in SEQ ID NO:1. SEQ ID NO: 7 does not share significant homology/identity with SEQ ID NO:1.

SEQ ID NOs: 1, 2, 5, and 6 (and the corresponding RNA and polypeptide sequences) are partial sequences of SEQ ID NO:175 of the Corixa (*infra*).

CORIXA Corp. (Corixa) (WO 98 37418)

The Office considers originally filed claims **1, 2, 11, 13-16** and **25-38** as lacking novelty in view of Corixa. The applicant disagrees, especially in view of the amendments herein.

Claims 1 and **2** as originally filed have been cancelled. Originally filed claim 11 (now renumbered to claim 5) has been amended to eliminate reference to SEQ ID NO: 1 and SEQ ID NO:2.

Originally filed **claims 13-14** (now renumbered to claims 6 and 8) have been amended to eliminate reference to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 5, and SEQ ID NO:6. Originally filed **claims 15-16** have been cancelled.

Originally filed **claims 25-26** (now renumbered to claims 13 and 14) have been amended to eliminate reference to SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO:13. Originally filed **claim 27** (now renumbered to claim 15), and originally filed **claims 28-32** (now renumbered to claims 16 and 20) by virtue of their dependence on originally filed claim 27 have been amended to eliminate reference to SEQ ID NO: 15, SEQ

ID NO: 16, SEQ ID NO: 19, and SEQ ID NO: 20. Originally filed **claim 33** (now renumbered to claim 21), and originally filed **claims 34-38** (now renumbered to claims 22-26) by virtue of their dependence on originally filed claim 27 have been amended to eliminate reference to SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 12, and SEQ ID NO: 13.

Therefore, amended claims **5, 6, 8, 13-26** (corresponding to amended originally filed claims 11,13-14, and 25-38) do not lack novelty in view of Corixa.

The Office further considers originally filed claims **1-38** as lacking an inventive step in view of Corixa. The applicant respectfully disagrees, especially in view of the amendments made herein. All of amended **claims 1-8** recite polynucleotides of SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 7, none of which are taught or suggested by Corixa. Furthermore, Corixa does not provide motivation to modify it's sequences in a manner to a arrive at the sequences of SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 7 as presently claimed.

Both SEQ ID NO: 3 and SEQ ID NO: 4 are splicing variants that are substantially different from SEQ ID NO:1. Both of SEQ ID NO: 3 and SEQ ID NO: 4 have sequence portions not found in the Corixa sequences, and SEQ ID NO: 3 and SEQ ID NO: 4 are therefore inconsistent with the Corixa sequences. One splicing variant of a parent sequence is not an obvious modification of a second splicing variant of the same parent sequence since there is no predictability where splicing will occur (*i.e.*, which splicing product will be produced), and under which condition certain splicing points will be utilized. Furthermore, it is well known in the art that different splicing variants may possess different biochemical functionality. With respect to SEQ ID NO: 7, the applicant points out that SEQ ID NO: 7 does not share a significant homology/identity with any of SEQ ID NO: 1 - SEQ ID NO: 6.

Each of amended **claims 9-14** and **21-26** recite polypeptides of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 14, none of which are taught or suggested by Corixa. Furthermore, Corixa does not provide motivation to modify it's sequences in a manner to a arrive at the sequences of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 14 as presently claimed.

Since the polypeptides of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 14 are the corresponding peptide to SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 7, respectively, the same arguments as above apply.

Each of amended **claims 15-20** recite polypeptides of SEQ ID NO: 17, SEQ ID NO: 18, or SEQ ID NO: 21, none of which are taught or suggested by Corixa. Furthermore, Corixa does not provide motivation to modify it's sequences in a manner to a arrive at the sequences of SEQ ID NO: 17, SEQ ID NO: 18, or SEQ ID NO: 21 as presently claimed.

Since the polynucleotides of SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 21 are the corresponding ribonucleotides to SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 7, respectively, the same arguments as above apply.

Therefore, amended claims **1-26** (corresponding to amended originally filed claims 5-7, 10-14, 19-21, and 25-38) do not lack an inventive step in view of Corixa.

Nelson et al. (Nelson) (PNAS (1999), Vol. 96, No. 6: 3114-3119)

The Office considers originally filed claim **15** as lacking novelty in view of *Nelson*. Originally filed claim 15 has been cancelled.

The Office further considers originally filed claims **1-38** as lacking an inventive step in view of *Nelson*. The applicant disagrees, especially in view of the amendments herein. Each of amended **claims 1-8** recite polynucleotides of SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 7. All of amended **claims 9-14** and **21-26** recite polypeptides of SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 14, and all of amended **claims 15-20** recite polynucleotides of SEQ ID NO: 17, SEQ ID NO: 18, or SEQ ID NO: 21.

With respect to each of the polynucleotides and/or polypeptides having SEQ ID NOs: 3, 4, 7, 10, 11, 14, 17, 18, and 21, the same arguments as above apply. There is simply no teaching, suggestion or motivation in the Nelson reference to modify Nelson's sequence to arrive at the SEQ IDs as presently claimed.

Moreover, amended claims **1-8, 10,** and **13-26** require that the polynucleotides of SEQ ID 3, 4, 7, 17, 18, and 21 ~~encode an intracellular polypeptide~~ and that the polypeptides with the SEQ ID 10, 11, and 14 are intracellular polypeptides. Nelson's sequence includes "...a 26 amino acid signal peptide with a cleavage site on the carboxyl side of Gly^{26}" (page 3116, third paragraph), and Nelson further teaches that "...[the]prostase is likely to be a secreted protein..." (page 3116, end of third paragraph). An intracellular polypeptide, however, is

entirely inconsistent with a secreted protein. Thus, *Nelson* teaches against the subject matter as presently claimed.

Therefore, amended claims 1-26 are not lacking an inventive step in view of *Nelson*.

Stephenson, et al. (Stephenson) (JBC (1999) Vol. 274; p23210-23214)

The Office regards claim 15 as lacking novelty in view of *Stephenson*. The publication date of *Stephenson* is August 13, 1999. The earliest priority date of the present application is May 20, 1999, which antedates the publication date of *Stephenson*. Therefore, *Stephenson* is not a proper reference.

Yousef, et al. (Yousef) (Cancer Res. (1999) Vol. 59, No. 17; p4252-4256)

The Office regards claim 15 as lacking novelty in view of *Yousef*. The publication date of *Yousef* is September 1, 1999. The earliest priority date of the present application is May 20, 1999, which antedates the publication date of *Yousef*. Therefore, *Yousef* is not a proper reference.

EP 0 936 270 A (BASF) (EP 0 936 270 A)

The Office regards claim 15 as lacking novelty in view of *BASF*. The publication date of *BASF* is August 18, 1999. The earliest priority date of the present application is May 20, 1999, which antedates the publication date of *BASF*. Therefore, *BASF* is not a proper reference.

WO 99 67384 A (Sprinzak) (WO 99 67384 A)

The Office regards claim 15 as lacking novelty in view of *Sprinzak*. The publication date of *Sprinzak* is December 29, 1999. The earliest priority date of the present application is May 20, 1999, which antedates the publication date of *Sprinzak*. Therefore, *Sprinzak* is not a proper reference.

Combinations

There is no combination of references that teaches, suggests, or provides motivation to modify a nucleotide or polypeptide sequence of a reference in a manner to arrive at the sequences as presently claimed.

Respectfully submitted,

Fahri Saatcioglu

Mailing Address:
Fish & Associates, LLP
c/o Fahri Saatcioglu
1440 N. Harbor Blvd., Suite 706
Fullerton, CA 92835 USA
Tel: 714-449-2337
Fax: 714-449-2339